THE JOURNAL OF EXPERIMENTAL MEDICINE

THE JOURNAL

OF

EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M.D.

VOLUME THIRTY-SECOND

WITH ONE HUNDRED PLATES AND SIXTY-ONE

FIGURES IN THE TEXT

NEW YORK

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

1920

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WAVERLY PRESS
THE WILLIAMS & WILKINS COMPANY
BALTIMORE, U. S. A.

JUL 1920

THE JOURNAL

OF

EXPERIMENTAL MEDICINE

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SIMON FLEXNER, M.D.

VOLUME XXXII, NO. 1 JULY 1, 1920

PUBLISHED MONTHLY

AT MOUNT ROYAL AND GUILFORD AVES., BALTIMORE, MD.

BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

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Entered as second-class matter June 3, 1916, at the Post Office at Baltimore, Md., under the act of March 3, 1879.
Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917.
Authorized June 29, 1918.

PUBLICATIONS OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

THE JOURNAL OF EXPERIMENTAL MEDICINE

EDITED BY

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STUDIES ON EXPERIMENTAL PNEUMONIA.

VII. Treatment of Experimental Pneumococcus Type I Pneumonia in Monkeys with Type I Antipneumococcus Serum.

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(*From the Bacteriological Laboratory of the Army Medical School, Washington.*)

(Received for publication, January 23, 1920.)

The treatment of Pneumococcus Type I pneumonia with Type I antipneumococcus serum has come into extensive use since the original studies in 1913 by Cole[1] and his coworkers. These investigators found that Pneumococcus Type I pneumonia, when not treated with serum exhibited a mortality rate of about 25 per cent. In a large series of serum-treated cases, however, they showed that the mortality rate could be reduced from this high figure to approximately 7 per cent. In spite of these convincing studies, Type I antipneumococcus serum has not come into such wide use as its efficacy would seem to justify. The reasons for this are several. Among them may be mentioned lack of familiarity with the subject, inexperience with the technique of administering serum, difficulty in obtaining a prompt determination of the type of pneumococcus, and finally a certain skepticism among many physicians as to the therapeutic value of serum treatment. Especially in the Army, in the various base hospitals where Type I antipneumococcus serum was used extensively, able clinicians were often heard to express doubt as to the usefulness of any kind of serum treatment in pneumonia.

The proper administration of Type I antipneumococcus serum in Pneumococcus Type I pneumonia has been followed, in the case of human beings, by excellent results, as many hospital records show. So far as known, however, no test of its value has ever been made in

[1] Cole, R., *J. Am. Med. Assn.*, 1913, lxi, 663; 1917, lxix, 505; *Arch. Int. Med.*, 1914, xiv, 56.

experimental pneumonia in animals when treated and untreated cases could be compared simultaneously. The studies of experimental pneumonia in monkeys which have been previously presented[2] show that pneumococcus pneumonia in monkeys differs in no essential respect either clinically or pathologically from pneumococcus pneumonia in man. The present study was undertaken, therefore, to determine by experiments on monkeys the therapeutic value of Type I antipneumococcus serum in Pneumococcus Type I pneumonia.

Method.

The Philippine *Macacus* (*Macacus syrichtus*) was used in all the experiments reported in this paper. Pneumococcus Type I pneumonia was induced in them, according to the method previously described, by the intratracheal injection of a highly virulent Pneumococcus Type I in amounts which experience had shown invariably produced a fatal pneumonia.

Type I antipneumococcus serum, prepared by the New York State Board of Health, was employed throughout. The serum, which was in no case administered until the animal had developed definite symptoms of pneumonia, was diluted in most instances with an equal quantity of normal salt solution, and slowly injected by means of a Luer syringe into the femoral vein. The dose employed was 10 cc. except in one experiment where doses of 20 cc. were given in order to determine the effect of large dosage. The dose of antipneumococcus serum recommended for man is 90 to 100 cc. As a Philippine *Macacus* weighs about one-sixteenth as much as man, the monkeys, in receiving only 10 cc., got a somewhat larger dose in proportion to their weight than a human being receives.

Effect of Type I Antipneumococcus Serum When Administered Early in the Disease.

Hospital patients suffering with Pneumococcus Type I pneumonia rarely receive their first injection of serum until 24 or 48 hours after the onset of the disease; in many cases even more time elapses before

[2] Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 403, 445, 499. Cecil, R. L., and Blake, F. G., *J. Exp. Med.*, 1920, xxxi, 519, 657, 685.

serum treatment is instituted. In the experiment about to be reported serum treatment was started 24 hours after the pneumococci were injected into the trachea of the monkey in order to determine the effect of antipneumococcus serum when administered early in the course of the disease. Monkey 94, which received 0.1 cc. of Pneumococcus Type I, was treated with serum. Monkey 93, which received only 0.001 cc. of Pneumococcus Type I, served as a control (Text-fig. 1).

Experiment 1.—Monkey 94 (Text-fig. 1). *Macacus syrichtus*, male; weight 4,602 gm.

May 12, 1919. Well and active.

May 14. Well and active.

May 15. Well and active. 10.35 a.m. Intratracheal injection of 0.1 cc. of 18 hour broth culture of Pneumococcus Type I (in 1 cc. of broth). 4.10 p.m. Quiet. Respirations moderately accelerated. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 43 colonies of Pneumococcus Type I.

May 16, 9.55 a.m. Appears moderately sick; huddled up on perch. Breathing rapid and labored. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 103 colonies of Pneumococcus Type I. 10 a.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution. No reaction. 3.55 p.m. Quiet. Breathing moderately accelerated. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. 4 p.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution. No reaction. 9 p.m. Quiet but appears in good condition; breathing easily. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. 9.30 p.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution (about half of material escaped into surrounding tissue).

May 17. Appears in good condition; breathing easily; temperature normal. 10.05 a.m. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. 10.10 a.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution. No reaction. 4 p.m. Monkey appears well; breathing normally. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution. No reaction.

May 18. Appears in good condition; breathing easily; temperature, however, has gone up. 11 a.m. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth.

May 19. Monkey appears in excellent condition; respiration quiet and slow; temperature still up, however. 11.45 a.m. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. 11.50 a.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution.

TEXT-FIG. 1, *a* and *b*. Experimental Pneumococcus Type I pneumonia treated with Type I antipneumococcus serum. (*a*) Monkey 94; treated with Type I antipneumococcus serum. (*b*) Monkey 93; untreated control.

No reaction. 4.30 p.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution.

May 20. Appears well and active. Temperature has fallen to normal by crisis.

May 21 to 26. Well and active.

May 28. Appears well and active. Slight rise in temperature during past 2 days may be due to serum disease though no other manifestations of the condition have appeared.

May 29. Well.

June 2. Continues well.

Monkey 93 (Text-fig. 1). *Macacus syrichtus*, male; weight 5,110 gm. Control for Monkey 94.

May 12, 1919. Well and active.

May 14. Well and active.

May 15. Well and active. 10.25 a.m. Intratracheal injection of 0.001 cc. of 18 hour broth culture of Pneumococcus Type I (in 1 cc. of broth). 4 p.m. Appears well. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 1 colony of Pneumococcus Type I.

May 16. Moderately sick; respirations increased. 10.10 a.m. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 337 colonies of Pneumococcus Type I.

May 17. Monkey sick; respirations rapid and labored; suggestive signs of involvement of right lower lobe. 10.20 a.m. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 500 colonies of Pneumococcus Type I.

May 18, 10.45 a.m. Condition the same; respirations rapid and labored. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 365 colonies of Pneumococcus Type I.

May 19. Sick; breathing rapidly; dullness and suppressed breathing over right lower lobe. 11.20 a.m. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 216 colonies of Pneumococcus Type I.

May 20. Appears sick; sitting huddled up; breathing rapidly. 11.35 a.m. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 24 colonies of Pneumococcus Type I.

May 21. Condition the same. 11.25 a.m. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 35 colonies of Pneumococcus Type I.

May 22. Sick; respirations rapid and labored. 4.15 p.m. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 73 colonies of Pneumococcus Type I.

May 23. Still sick; respirations rapid and grunting; marked dullness over entire right chest, and bronchial breathing. 10.55 a.m. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 66 colonies of Pneumococcus Type I.

May 24. Condition the same. 10.45 a.m. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 12 colonies of Pneumococcus Type I.

May 25. Still sick; respirations rapid and grunting. 10.30 a.m. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 3 colonies of Pneumococcus Type I.

May 26. Seems better but still sick. 10.20 a.m. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 46 colonies of Pneumococcus Type I.

May 27. Appears very sick; lying on floor of cage most of time. 11.20 a.m. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 750 colonies of Pneumococcus Type I. 3.15 p.m. Died.

Autopsy.—May 27, 4 p.m., immediately after death.

Anatomical Diagnosis.—Experimental lobar pneumonia (Pneumococcus Type I), right upper, middle, and lower lobes; acute fibrinous pleuritis, right side; Pneumococcus Type I septicemia; hypertrophy and dilatation of the heart; cloudy swelling of viscera.

Subject.—Large male *Macacus*. Body is still warm. Abdominal organs normally disposed; liver and kidneys show cloudy swelling. Pericardial and left pleural cavities are normal. There are fresh fibrinous adhesions throughout the right pleural cavity.

Heart.—Markedly enlarged; the cavities on both the right and left sides show considerable dilatation. The heart muscle and valves appear normal. *Left lung.*—Somewhat collapsed, pale, and crepitant throughout. *Right lung.*—Entire lung shows a massive consolidation. It is considerably larger and heavier than the left lung, and its surface is covered with dark red fibrinous tags. Cut surface of right lung shows typical gray hepatization. *Trachea and bronchi.*—Filled with frothy mucus. *Bronchial lymph glands.*—Enlarged.

Microscopic Examination.—Right lower lobe.—A considerable part of the section is necrotic. Inside the necrotic areas there are irregular conglomerations of polymorphonuclear leucocytes, many of them undergoing necrosis and disintegration. In the portion of the section which is still preserved, the tissue shows the typical picture of resolution associated with early organization. There are swelling and desquamation of the alveolar epithelium with plugs of hyaline material in many of the alveoli. A few poorly staining pneumococci are found in the section. *Bronchial lymph glands.*—Sinuses contain large macrophage cells and a moderate number of lymphoid cells.

Autopsy Cultures.—Heart's blood, Pneumococcus Type I; right bronchus, abundant colonies of Pneumococcus Type I; right lower lobe, few colonies of Pneumococcus Type I.

These two protocols indicate that both monkeys promptly developed pneumonia following intratracheal inoculation with Pneumococcus Type I. In Text-fig. 1 it will be seen that both monkeys

presented sharp temperature and leucocyte reactions and both developed positive blood cultures. The institution of serum treatment, however, in Monkey 94 had a marked effect on the temperature which dropped almost immediately to normal. Moreover, the blood culture which had shown 103 colonies per 0.5 cc. of blood promptly became sterile following the first injection of 10 cc. of serum. The importance of keeping up serum treatment even after the temperature has dropped is well shown in this experiment. When the temperature had been practically normal for 2 days, it was decided to discontinue the serum treatment. On the following morning, however, the temperature was at 103.2°F., and on the next day it rose to 104.5°. Serum treatment was again started and the temperature dropped by crisis from 104.5° to 100° and remained normal thereafter.

There can be no reasonable doubt that in this case serum treatment greatly shortened the course of the disease and saved the monkey's life. It received 0.1 cc. of broth culture of Pneumococcus Type I, a dose which has, in our experience, been invariably fatal. The control monkey received only 0.001 cc. of broth culture and died on the 13th day of the disease. This experiment also shows that serum treatment when administered early promptly removes pneumococci from the blood, and shortens the course of the disease.

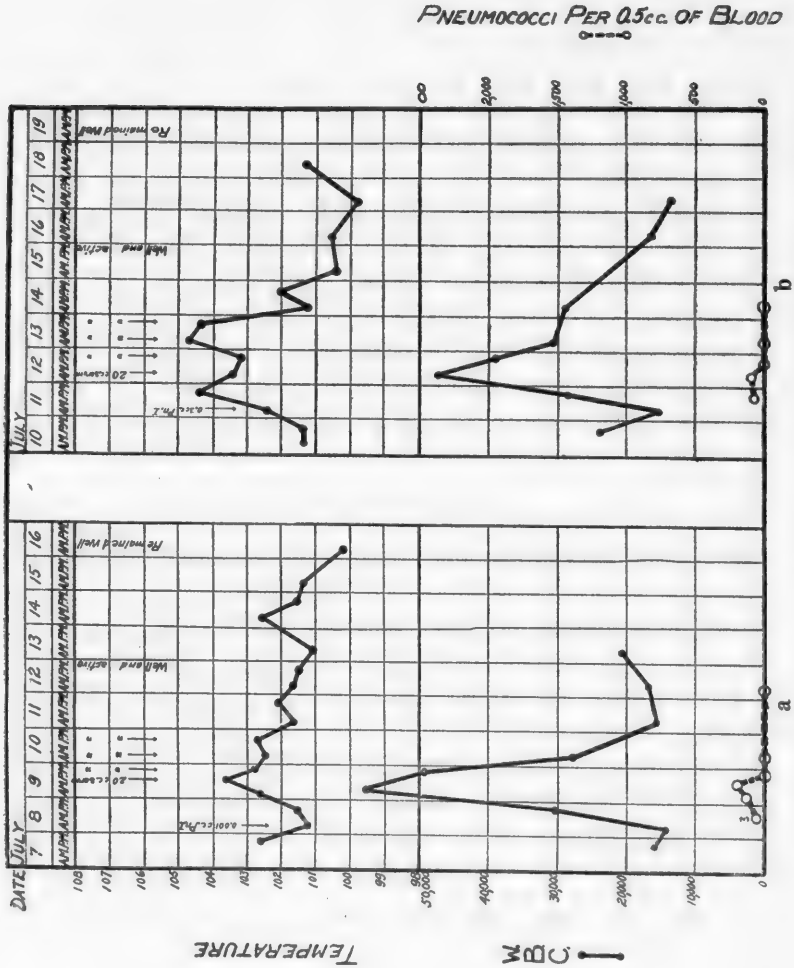
Effect of Large Doses of Serum Administered Early in the Disease.—The object of the following experiment was to produce an abortive form of pneumonia by frequent injections of large doses of serum early in the disease.

Experiment 2.—Monkey 45 (Text-fig. 2). *Macacus syrichtus*, male; weight 4,544 gm.

July 7, 1919. Well and active.

July 8. Well and active. 10 a.m. Intratracheal injection of 0.001 cc. of 18 hour broth culture of Pneumococcus Type I (in 1 cc. of broth). 3.50 p.m. Seems well. Blood culture. 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 3 colonies of Pneumococcus Type I.

July 9, a.m. Having a severe chill; respirations moderately accelerated. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 37 colonies of Pneumococcus Type I. 3 p.m. Sitting huddled up with rapid grunting respiration; evidently in pain; marked hyperesthesia in left axilla. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 111



TEXT-FIG. 2, *a* and *b*. Abortive Pneumococcus Type I pneumonia, produced by early administration of large doses of Type I antipneumococcus serum. (*a*) Monkey 45. (*b*) Monkey 79.

colonies of Pneumococcus Type I. 3.05 p.m. Intravenous injection of 20 cc. of Antipneumococcus Serum Type I. 9 p.m. Appears better but still breathing rapidly. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. 9.05 p.m. Intravenous injection of 20 cc. of Antipneumococcus Serum Type I.

July 10. Quiet but otherwise appears well. Breathing easily. 10 a.m. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. 10.05 a.m. Intravenous injection of 20 cc. of Antipneumococcus Serum Type I. 3.45 p.m. Quiet but otherwise appears well. Intravenous injection of 20 cc. of Antipneumococcus Serum Type I.

July 11. Well and active; breathing easily; appears to have completely recovered.

July 12. Appears well and active. 10.20 a.m. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth.

July 13 to 16. Well and active.

Monkey 79 (Text-fig. 2). *Macacus syrichtus*, male; weight 6,425 gm.

July 10, 1919. Well and active.

July 11. Well and active. 10.10 a.m. Intratracheal injection of 0.3 cc. of 18 hour broth culture of Pneumococcus Type I. 4 p.m. Quiet. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 11 colonies of Pneumococcus Type I.

July 12. Appears moderately sick; breathing rapidly. 9.55 a.m. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 15 colonies of Pneumococcus Type I. 10 a.m. Intravenous injection of 20 cc. of Antipneumococcus Serum Type I. Vomited 5 minutes later. 3.45 p.m. Appears better; breathing quieter. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. 4 p.m. Intravenous injection of 20 cc. of Antipneumococcus Serum Type I. No reaction.

July 13. Quiet; refuses food; breathing moderately accelerated. 10.30 a.m. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. 10.35 a.m. Intravenous injection of 20 cc. of Antipneumococcus Serum Type I. 6 p.m. Intravenous injection of 20 cc. of Antipneumococcus Serum Type I.

July 14. Temperature has fallen to normal by crisis. Appears well and active.

July 15 to 18. Well and active.

Both the monkeys in this experiment presented symptoms of pneumonia following the intratracheal injection of Pneumococcus Type I. In both there were sharp temperature and leucocyte reactions and both monkeys promptly developed positive blood cultures. In Monkey 45 serum treatment was started 29 hours after intratracheal injection. Reference to Text-fig. 2 shows that the object of the experiment was attained; namely, an abortive pneumonia. The tempera-

ture immediately returned to normal, the blood culture became sterile 6 hours after the injection of serum, and 4 days after intratracheal injection the monkey was entirely well and remained so. Monkey 79 received a much larger dose of culture (0.3 cc.) than Monkey 45 received, and for that reason the infection was not so promptly overcome as in Monkey 45. Even here, however, four large doses of serum were sufficient to bring the temperature down to normal, where it remained permanently. The blood culture followed the usual course in serum-treated animals and was sterile 6 hours after serum treatment was started. This experiment shows that by doubling the size of the dose serum treatment was made even more rapidly efficacious than in Experiment 1. An abortive type of pneumonia was produced in which the acute symptoms lasted only 24 to 48 hours.

Effect of Type I Antipneumococcus Serum When Administered Late in the Disease.

In the two preceding experiments the value of early serum treatment has been demonstrated. In the following experiments it was desired to determine the effect of serum treatment when initiated late in the disease. In the first monkey (No. 99) serum treatment was not started until the 3rd day of the disease, in the second monkey (No. 113) not until the 5th day. Both monkeys received doses of culture (0.01 cc.) which without serum treatment had invariably proved fatal. The control (Monkey 112) received the same dose of culture intratracheally and was treated with normal horse serum (Text-fig. 3).

Experiment 3.—Monkey 99 (Text-fig. 3). *Macacus syrichtus*, male; weight 6,020 gm.

June 4, 1919. Well and active.

June 5. Well and active. 10.30 a.m. Intratracheal injection of 0.01 cc. of 18 hour broth culture of Pneumococcus Type I (in 1 cc. of broth). 4.30 p.m. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 26 colonies of Pneumococcus Type I.

June 6, 10.30 a.m. Moderately sick; respirations rapid. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 650 colonies of Pneumococcus Type I.

June 7. Sick; respirations rapid and labored. 9.55 a.m. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 800 colonies of Pneumococcus Type I. 10 a.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution. 3.55 p.m. No definite improvement. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. 4 p.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution. 9.15 p.m. Still sick but not so toxic. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. 9.20 p.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution.

June 8. Temperature still elevated but monkey appears better; respirations moderately accelerated. 10.30 a.m. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. 10.35 a.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution. 4.30 p.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution.

June 9. Marked improvement; seems fairly well; respirations only moderately accelerated; temperature still elevated, however. 10.15 a.m. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. 10.20 a.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution. 4 p.m. Condition the same. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution. 9 p.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution.

June 10. Appears in good condition but moderately sick. 10 a.m. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. 10.15 a.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution. 4.15 p.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution. 9.15 p.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution.

June 11. Improving; more active; respirations still accelerated; dullness over right lower lobe. 10 a.m. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. 10.05 a.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution. 4.05 p.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution.

June 12. Shows marked improvement; respirations easy; temperature falling. 10 a.m. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. 10.05 a.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution. 4 p.m. Temperature normal; condition excellent. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution.

June 13, 16, 18, 24. Continues well.

Monkey 113 (Text-fig. 3). *Macacus syrichtus*, male; weight 5,285 gm.

June 4, 1919. Well and active.

June 5. Well and active. 10.45 a.m. Intratracheal injection of 0.01 cc. of 18 hour broth culture of Pneumococcus Type I (in 1 cc. of broth). 4.45 p.m. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, 1 colony of Pneumococcus Type I.

June 6, 10.40 a.m. Moderately sick; respirations rapid. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 88 colonies of Pneumococcus Type I.

June 7. Sick; respirations rapid and labored. 10.30 a.m. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 400 colonies of Pneumococcus Type I.

June 8. Sick; respirations rapid and labored. 11 a.m. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 69 colonies of Pneumococcus Type I.

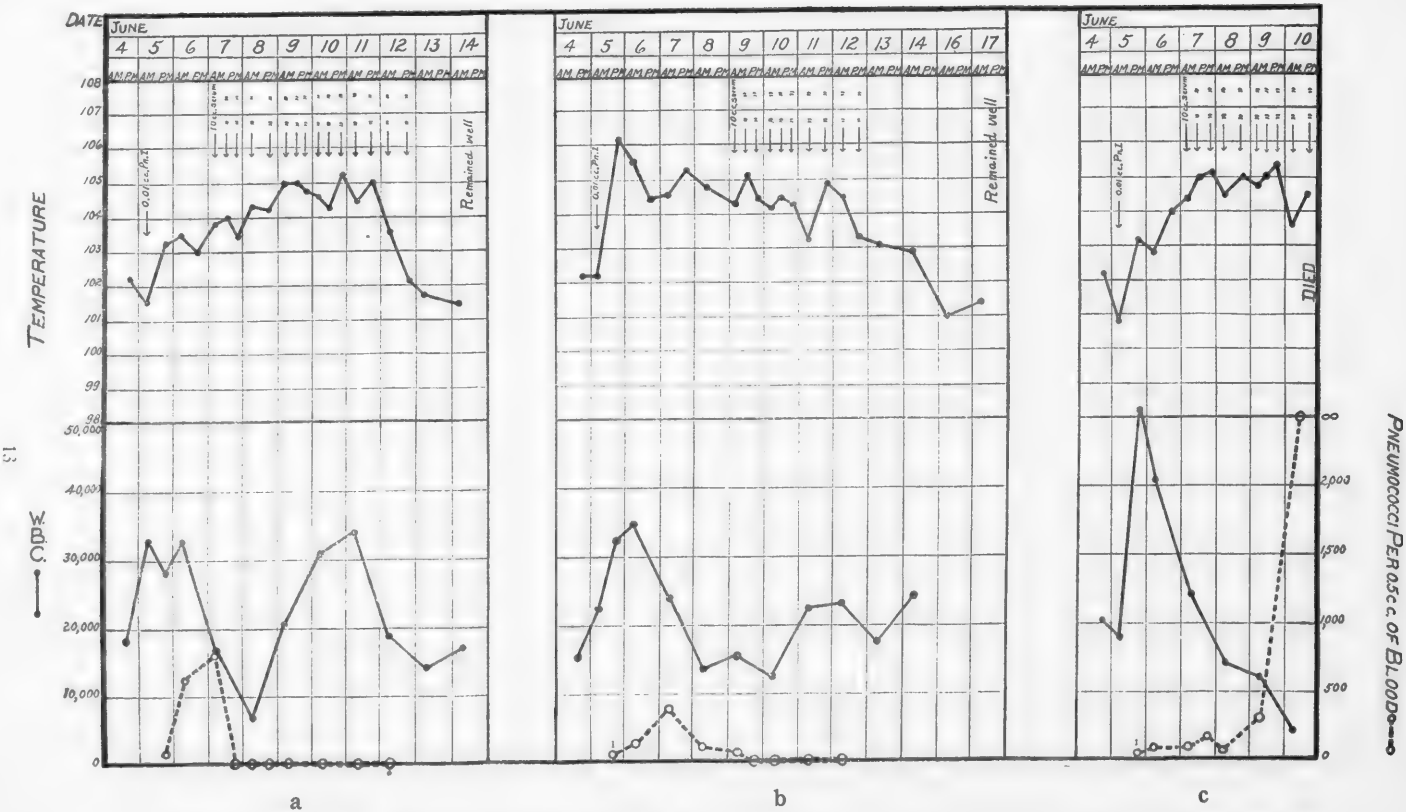
June 9. Sick; respirations rapid and labored. 10.25 a.m. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 17 colonies of Pneumococcus Type I. 10.30 a.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution. 4.15 p.m. Appears better. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. 4.20 p.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution. 9.30 p.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution.

June 10. Appears better and fairly active; temperature still elevated and respirations moderately rapid. 10.15 a.m. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. 10.20 a.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution. 4.20 p.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution. 9.30 p.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution.

June 11. Monkey appears much better; respirations still slightly accelerated. 10.15 a.m. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. 10.20 a.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution. 4.20 p.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution.

June 12. Appears in good condition. 10.15 a.m. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. 10.20 a.m. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution. 4.10 p.m. In excellent condition; temperature falling. Intravenous injection of 10 cc. of Antipneumococcus Serum Type I plus 10 cc. of normal saline solution.

June 13, 14, 17, 24. Remains well.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TEXT-FIG. 3, *a*, *b*, and *c*. Experimental Pneumococcus Type I pneumonia, showing the effect of Type I antipneumococcus serum when started late in the disease. (*a*) Monkey 99; treated with Type I antipneumococcus serum. (*b*) Monkey 113; treated with Type I antipneumococcus serum. (*c*) Monkey 112 (control); treated with normal horse serum.

These two protocols show that even when serum treatment is started late in the disease the life of the monkey may still be saved. The number of injections necessary, however, to achieve success was much larger than in the previous experiments in which treatment was started earlier. Monkey 99 received 0.01 cc. of broth culture of Pneumococcus Type I intratracheally and promptly developed pneumonia. 48 hours after the inoculation serum treatment was started, in injections of 10 cc. each, administered two or three times each day. This treatment was kept up for 6 days before the temperature finally became normal. It will be seen, however, that in this experiment as in the previous ones, the blood, which was heavily infected when treatment was started, became immediately sterile after the institution of serum treatment. In Monkey 113 the procedure was the same as with Monkey 99, except that serum treatment was delayed until 96 hours (4 days) after the intratracheal injection of pneumococci. Serum treatment was continued 4 days before the temperature returned to normal. As the infecting dose in both these monkeys was well above the minimum lethal dose, there is every reason to believe that both monkeys would have died from the infection if serum treatment had not been employed. This opinion is well supported by the death of the control (Monkey 112). These experiments show that with delay in the institution of serum treatment so prompt a cure cannot be expected as when treatment is started early in the disease, and they emphasize, therefore, very strongly the importance and value of early treatment.

Effect of Normal Horse Serum.—The control in this experiment (Monkey 112) was treated with normal horse serum in order to determine whether non-specific factors played any part in the favorable results obtained with antipneumococcus serum. The monkey received 0.01 cc. of broth culture of Pneumococcus Type I intratracheally, and 48 hours later serum treatment was started, the injections paralleling those of Monkey 99.

Monkey 112 (Text-fig. 3). *Macacus syrichtus*, male; weight 3,975 gm.

June 4, 1919. Well and active.

June 5. Well and active. 10.50 a.m. Intratracheal injection of 0.01 cc. of 18 hour broth culture of Pneumococcus Type I (in 1 cc. of broth). 4.50 p.m. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 1 colony of Pneumococcus I.

June 6, 10.45 a.m. Moderately sick; respirations accelerated. Blood culture: 0.5 cc. in broth, Pneumococcus I; 0.5 cc. in agar plate, 52 colonies of Pneumococcus I.

June 7, 10.20 a.m. Monkey sick; respirations rapid and labored. Blood culture: 0.5 cc. in broth, Pneumococcus I; 0.5 cc. in agar plate, 51 colonies of Pneumococcus I. 10.22 a.m. Intravenous injection of 10 cc. of normal horse serum plus 10 cc. of normal saline solution. No reaction. 4.10 p.m. Blood culture: 0.5 cc. in broth, Pneumococcus I; 0.5 cc. in agar plate, 90 colonies of Pneumococcus I. 4.15 p.m. Intravenous injection of 10 cc. of normal horse serum plus 10 cc. of normal saline solution. 9.30 p.m. Sick; respirations rapid and labored. Blood culture: 0.5 cc. in broth, Pneumococcus I; 0.5 cc. in agar plate, 21 colonies of Pneumococcus I. 9.35 p.m. Intravenous injection of 10 cc. of normal horse serum plus 10 cc. of normal saline solution.

June 8. Sick; respirations rapid and grunting. 10.40 a.m. Blood culture: 0.5 cc. in broth, Pneumococcus I; 0.5 cc. in agar plate, 17 colonies of Pneumococcus I. 10.45 a.m. Intravenous injection of 10 cc. of normal horse serum plus 10 cc. of normal saline solution. 5 p.m. Intravenous injection of 10 cc. of normal horse serum plus 10 cc. of normal saline solution.

June 9. Very sick; respirations rapid and labored; dullness, bronchial breathing, and râles throughout right axilla. 10.45 a.m. Blood culture: 0.5 cc. in broth, Pneumococcus I; 0.5 cc. in agar plate, 305 colonies of Pneumococcus I. 10.50 a.m. Intravenous injection of 10 cc. of normal horse serum plus 10 cc. of normal saline solution. 4.30 p.m. Very sick. Intravenous injection of 10 cc. of normal horse serum plus 10 cc. of normal saline solution. 10 p.m. Very sick. Intravenous injection of 10 cc. of normal horse serum plus 10 cc. of normal saline solution.

June 10. Very sick; respirations rapid and labored. 10.30 a.m. Blood culture: 0.5 cc. in broth, Pneumococcus I; 0.5 cc. in agar plate, innumerable colonies of Pneumococcus I. 10.35 a.m. Intravenous injection of 10 cc. of normal horse serum plus 10 cc. of normal saline solution. 4.30 p.m. Intravenous injection of 10 cc. of normal horse serum plus 10 cc. of normal saline solution. 8.30 p.m. Died.

Autopsy.—Performed June 11, 9.30 a.m.

Anatomical Diagnosis.—Experimental lobar pneumonia (Pneumococcus Type I); red and gray hepatization of right middle and lower lobes; engorgement of left lower lobe; acute fibrinous pleuritis, right side; hypertrophy of the heart; cloudy swelling of viscera; Pneumococcus Type I septicemia.

Subject.—Large male *Macacus*. Postmortem rigidity is present. Abdominal organs show cloudy swelling but otherwise are normal. Pericardial and left pleural cavities are normal. There are a few fine fresh adhesions over the right lower lobe.

Heart.—Definitely enlarged and left ventricular wall appears thicker than normal. Myocardium and valves show no changes. *Right lung.*—Considerably

larger than the left, due to massive consolidation of the middle and lower lobes, and emphysema of upper lobe. The latter is pale and air-containing throughout. The middle and lower lobes are completely consolidated and are plastered together by fibrin. There is also a fibrinous exudate on the surface. The cut surface of the consolidated lobes is pinkish gray, mottled with patches of reddish hue, indicative of the transition from red to gray hepatization. *Left lung.*—Air-containing throughout, but the posterior portion shows marked hypostatic congestion. This is most marked in the lower lobe, the greater part of which presents a dark red color. A part of this congestion may be an expression of early infection; that is, of engorgement. *Trachea and bronchi.*—Contain blood-tinged mucus.

Microscopic Examination.—Right lower lobe.—Section shows an exudate of polymorphonuclear leucocytes which almost completely fills the alveoli. In some places the exudate also contains many red blood cells. In a few places resolution is beginning, as is indicated by the desquamation of epithelium and the disintegration of the leucocytes. Sometimes the dissolution of the cells is so advanced that practically nothing remains but an amorphous residue. There is little or no perivascular infiltration of leucocytes, but there is a very noticeable extravasation of red blood cells in the perivascular tissue. The bronchi contain pus; the bronchial epithelium has been destroyed in some places. Section stained for bacteria shows a moderate number of pneumococci in the alveoli. Many of these, however, stain poorly. *Left lower lobe.*—Section shows engorgement of all the blood vessels, including the capillaries. There is a moderate amount of interstitial infiltration of leucocytes in some places. A few pneumococci are seen here and there in the alveolar walls.

Autopsy Cultures.—Heart's blood, Pneumococcus I; right lower lobe, Pneumococcus I.

It is obvious from a study of this protocol that normal horse serum had no beneficial effect whatever on the pneumococcus septicemia, which gradually increased in severity; nor did it have any apparent effect on the course of the pneumonia. The monkey died on the 6th day of the disease, in spite of ten injections of horse serum.

DISCUSSION.

Little discussion is needed in connection with the experiments which have just been described. They prove without any reasonable doubt that Type I antipneumococcus serum has a highly specific therapeutic action on Pneumococcus Type I pneumonia. This action is most rapidly effective when the serum is administered early in the disease. At this stage intravenous injection of Type I antipneumo-

coccus serum at frequent intervals will completely abort what would otherwise be a serious and often fatal disease. These experiments also show that even when started late in very severe cases serum treatment may cure the disease, though without appreciably shortening its course.

Failure to obtain favorable results with antipneumococcus serum can usually be charged to one or more of several causes: (1) Incorrect determination of the type of pneumococcus. Pneumococcus Type I serum is highly specific for Pneumococcus Type I pneumonia, and probably has no beneficial effect whatever when used in other types of pneumonia. (2) The serum treatment is started too late in the disease. This is one of the most frequent mistakes made in connection with serum treatment of pneumonia. When this delay is unavoidable, however, the present studies have shown that it is always worth while to use the serum late in the disease, though in such cases a larger number of injections may be necessary. (3) The serum is administered in too small doses. This mistake was often made in the Army camp hospitals where only 40 or 50 cc. of serum were injected at one time. (4) The serum is not given at sufficiently frequent intervals. Cole recommends that serum be administered every 8 hours. As a matter of practice, however, in many hospitals, serum is given only once or twice in 24 hours and sometimes not that often.

In the summer of 1918 there was a small epidemic of Pneumococcus Type I pneumonia among the negro troops at Camp Wheeler in which the mortality rate was surprisingly high. In a series of twenty-nine cases of Pneumococcus Type I pneumonia that received Type I antipneumococcus serum, there were ten deaths, or a mortality rate of 34.5 per cent. As the mortality rate of Type I pneumonia in cases treated with serum was only 7 per cent in the Rockefeller Institute series, it was evident that there was some defect in the method of administering the serum at Camp Wheeler. An investigation made by one of us showed that two-thirds of these cases did not receive serum until 48 hours or more after admission to the hospital. One-half of the cases did not receive the first injection of serum until at least 4 days after admission. The average number of serum injections was three, and the total amount of serum given

each patient averaged about 175 cc. From these figures it would appear that at Camp Wheeler antipneumococcus serum was not administered early enough, frequently enough, or in sufficiently large doses, to be of therapeutic value.

Finally, it should be pointed out that two of the monkeys in the experiments (Nos. 45 and 79) received doses of serum about three times as large proportionately as human patients receive in our hospitals, and suffered no apparent ill effects therefrom. This brings up the question whether even more brilliant results could not be obtained with serum treatment in man, if the routine dose of antipneumococcus serum were larger. It would have to be determined, of course, whether this modification could be introduced without discomfort or danger to the patient.

CONCLUSIONS.

1. In experimental Pneumococcus Type I pneumonia in monkeys the intravenous injection of Type I antipneumococcus serum exercises a specific therapeutic effect, frees the blood promptly and permanently from pneumococci, shortens the course of the disease, and greatly moderates its severity. Of five monkeys inoculated intratracheally with lethal doses of Pneumococcus Type I, all developed pneumonia, and all recovered following the administration of Type I antipneumococcus serum, while the controls died.

2. The earlier the serum is administered the shorter and less severe the pneumonia. Frequent injections are also an important factor in obtaining favorable results. When serum treatment is instituted late in the disease, the injections must usually be continued over a longer period of time in order to achieve success.

3. Normal horse serum exerts no beneficial effect whatever in experimental Pneumococcus Type I pneumonia.

A GROUP OF PARATYPHOID BACILLI FROM ANIMALS CLOSELY RESEMBLING THOSE FOUND IN MAN.

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(Received for publication, March 5, 1920.)

In a previous paper (1) the writer described a group of paratyphoid bacilli, isolated from animals, that with the usual cultural methods seemed to be the same as paratyphoid B, or *Bacillus schottmülleri*, as Winslow, Kligler, and Rothberg (2) suggest we call the latter organism. Serologically they could be separated from one another by agglutination absorption tests and to a certain extent by the type of clumps formed in immune sera. Five new cultures belonging to this group have recently been studied and a further effort has been made to differentiate them from the human paratyphoids by a method less complicated than agglutination absorption.

Five of the cultures studied came from swine infected with hog-cholera virus, two came from guinea pigs, and one each from a child, a cow, a pigeon, and a mouse. Four cultures of *Bacillus schottmülleri* obtained from Dr. C. Krumwiede were used for comparison. The date of isolation and other facts regarding the above cultures are given in the paper already referred to. In addition, cultures isolated by Dr. Smith in 1918 from five calves have been studied and found to belong to the same group, and still more recently additional cultures of this group have been isolated from swine infected with hog-cholera virus. It is evident that these organisms are quite widespread among our domestic animals but just what relation they have to animal diseases will require much further study. In hog-cholera they may tend to emphasize the intestinal lesions, but cases of this disease occur with extensive intestinal changes from which organisms of this group cannot be isolated.

Cultural Studies.

Sixteen cultures of the animal paratyphoids have been studied culturally and they have all corresponded to one another and to *Bacillus schottmülleri*. The results of the study are given in Table I, which includes the characters of *Bacillus enteritidis* and of the hog-cholera bacillus.

Some of the strains were tested with inosite with unsatisfactory results as the amount of acid produced by the paratyphoids was so small that it seemed possible that it was due to impurities. Other carbohydrates might have been included in the study but they are so

TABLE I.

Cultural Characters of Paratyphoids Studied.

Organisms.	Motility.	Gram stain.	Indole.	Hydrogen sulfide.	Gelatin liquefied.	Dextrose.	Maltose.	Mannitol.	Xylose.	Dulcite.	Arabinose.	Lactose.	Saccharose.	Salicin.	Glycerol.	Raffinose.	Dextrin.
B. schottmülleri (paratyphoid B)	+	−	0	+	0	AG*	AG	AG	AG	AG	AG	0	0	0	0	0	0
B. enteritidis	+	−	0	+	0	"	"	"	"	"	"	0	0	0	0	0	0
Animal paratyphoids	+	−	0	+	0	"	"	"	"	"	"	0	0	0	0	0	0
Hog-cholera bacilli	+	−	0	0	0	"	"	"	"	0	0	0	0	0	Slightly acid.	0	0

* AG indicates acid and gas formation.

expensive that they would be of no practical value in differentiating the two groups. As shown in the table, no cultural differences have been found between *Bacillus enteritidis*, *Bacillus schottmülleri*, and the animal paratyphoids.

In the hope that a difference might be detected between animal and human paratyphoids, by a study of the hydrogen ion concentration, cultures were made in dextrose and xylose broth. Both groups gave a hydrogen ion concentration of pH 5.0 in dextrose broth after 4 days incubation, while in xylose broth the pH varied from 5.6 to 5.2. Some of the animal strains acted more slowly and others apparently utilized more of the carbohydrate than did the human strains. The difference is so slight, however, that it is of no value in classification.

Serological Studies.

When living cultures are used for immunization of rabbits the sera produced will usually agglutinate both the human and animal paratyphoids to the same titer limit. At times, however, one gets a serum that will agglutinate bacilli of the group used in the immunization in higher dilutions than it does those of the other group. This is apparently due to the individual rabbit rather than the bacteria, for the same culture will act differently in different rabbits.

Cultures from both human and animal sources are agglutinated in only the lower dilutions of sera from animals immune to *Bacillus enteritidis* and as the results obtained in the present study do not differ from those previously given they need not be repeated here.

When 24 hour bouillon cultures are used as antigens the clumps formed by bacilli of the same group as the immunizing strain are flocculent and after 2 hours incubation form a mass occupying from one-quarter to one-half the column of liquid. Bacilli of the other group as a rule give very compact clumps which after standing in the refrigerator over night form a thin film on the bottom of the test-tube. This difference in the clumping is striking but unfortunately it does not always hold true. If growth is more abundant than the average, flocculent clumping may occur where a compact type is expected. The amount of dextrose in the bouillon influences the type of clumps formed. Apparently the amount of growth rather than the change in reaction is responsible for the change. As a rule, however, one can differentiate fairly well between the two groups by the type of clumps, but it can only be regarded as a tentative means of differentiation.

When heated or formalinized bouillon cultures or suspensions in salt solution of the growth from agar slants are used as antigens, agglutination will occur in the same dilutions of serum and the clumps are so nearly alike in character that the two groups cannot be differentiated.

When formalinized bouillon cultures are standardized and agglutinated according to Dreyer's method (3) with the readings made after 2 hours incubation at 50°C. in the water bath and 15 minutes at room temperature, the two groups cannot be differentiated by the degree of agglutination or the type of clumps.

Numerous agglutination absorption tests have been made and the results have confirmed those previously reported. The animal strains will absorb from *Bacillus schottmülleri* sera the agglutinin for all the

TABLE II.

Summary of Absorption Experiments with Serum of Rabbit 9, Immunized to Calf-Typhus V by Injection of Living Cultures.

Culture tested.	Titer limit of serum saturated with.					
	Nothing; *i.e.*, control.	*B. schottmülleri* 232.	*B. schottmülleri* 242.	Calf-typhus V.	Swine-typhus I.	Swine-typhus V.
Calf-typhus I	51,200	25,600	25,600	—	—	—
" III	51,200	—	—	—	400	400
" IV	51,200	25,600	25,600	800	—	—
" V	51,200	25,600	25,600	200	400	400
Swine-typhus I	51,200	12,800	12,800	—	200	—
" V	51,200	25,600	25,600	—	—	200
Pigeon-typhus	51,200	25,600	25,600	—	—	—
Mouse-typhus I	51,200	25,600	25,600	—	—	—
Hog-cholera XII	51,200	400	200	—	—	—
" XIII	51,200	400	200	—	—	—
B. schottmülleri 232	51,200	400	200	100	—	—
" " 242	51,200	400	400	—	—	—
" " 225	51,200	200	800	100	—	—

TABLE III.

Absorption of Agglutinins from B. schottmülleri Serum by Calf-Typhus Cultures.

Culture tested.	Titer limit of serum of Rabbit 10, immune to *B. schottmülleri* 232, absorbed with.		
	Nothing; *i.e.*, control.	Calf-typhus V.	Calf-typhus I.
Calf-typhus I	12,800	200	400
" IV	12,800	100	100
" V	12,800	200	400
Swine-typhus I	12,800	200	400
B. schottmülleri 225	25,600	25,600	25,600
" " 232	25,600	25,600	25,600

animal cultures without removing those for the human cultures, and, *vice versa*, *Bacillus schottmülleri* will remove from the sera of animals immune to the animal cultures the agglutinin for the human cultures

and will not remove those for the animal strains. The calf and swine cultures are identical in this respect as is shown in Tables II and III.

While the great majority of the agglutination tests was made with sera of animals immunized by the injection of living bouillon cultures, the possibility that sera produced by the injection of heated cultures might differentiate the two groups has also been considered. The serum of an animal immunized by three injections of a suspension of agar slant growth, killed by heating to 60°C. for 1 hour, agglutinated both groups of bacilli to the same degree. When sera of animals immunized by bacilli heated to 70° for 1 hour were tested, it was found that the bacilli of the immunizing group were agglutinated in a somewhat higher dilution than were those of the other group. The difference was not great enough to make it a valuable means of differentiation. Better results might be obtained by using heated and washed bacilli, but this has not been tried.

Since the type of clumping indicates that the difference in these two groups lies in the flagella, antigens were prepared by shaking suspensions of the bacilli for a short time, centrifugalizing, and using the supernatant fluid for precipitation and complement fixation tests. When the antigen belonged to the same group as the immune serum, the precipitate was more flocculent than when it belonged to the other group, but the amount of precipitation was about the same. When such antigens were used for complement fixation, the inhibition of hemolysis was the same with sera of rabbits immune to either group. Formalinized bouillon cultures used as antigens in complement fixation tests were likewise of no value in differentiating the two groups. These results agree with those of the previous paper where extracts of the bacilli were used as antigens.

The results of the serological tests show that the animal paratyphoids which are usually agglutinated to the titer limit in sera of animals immune to *Bacillus schottmülleri* can best be separated from the latter by agglutination absorption tests. With the methods commonly used in agglutination tests they would be classed as *Bacillus schottmülleri.*

Cross-Immunization Tests.

In the previous paper it was noted that the injection of living cultures of the swine paratyphoids immunized rabbits to a virulent hog-cholera bacillus, whereas rabbits treated in the same way with living cultures of human paratyphoid were not immune. This seemed at that time to indicate that the swine cultures were more closely related to, or possibly were a variety of, the hog-cholera bacillus. These tests have been repeated and the results are given in Table IV.

It will be seen that the calf cultures also immunize rabbits to the hog-cholera bacillus, whereas the human cultures do not. Examination of the records shows that the injection of the animal cultures produces a more severe type of reaction than does the injection of the human cultures. The local lesion is larger and the rise in temperature following a subcutaneous reaction is higher.

Two rabbits were each given a subcutaneous injection of 0.1 cc. of a 24 hour bouillon culture of two strains of human paratyphoid and were chloroformed 1 week later. There was a slight local lesion from which the organisms injected were cultured. The spleen and other organs were normal and cultures from as much as 0.5 cc. of blood, a piece of liver as large as a pea, and stab cultures from the spleen were sterile. Pea-sized bits of spleen from one rabbit showed no organisms, while from the other there was a growth which proved to be due to the organism injected. 1 week after an intravenous injection of 0.1 cc. of 24 hour bouillon cultures, two other rabbits were chloroformed and their spleens were found to be slightly enlarged. Cultures from the spleen of one of these rabbits showed a growth due to the organism injected, whereas the spleen of the other animal failed to show such organism. The blood, liver, and bile of both rabbits were sterile.

These results show that there is very little growth of the human paratyphoid bacilli in rabbits when they are used in the same amounts that will produce a general invasion by the animal cultures. It therefore seems probable that the immunity to the hog-cholera bacillus produced by the animal paratyphoids is due to the fact that they multiply in the body and increase the resistance enough so that the animals are able to withstand the hog-cholera bacilli injected, when the amount of the latter used is about ten times the minimal lethal dose.

TABLE IV.

Test of Power of Various Paratyphoids to Immunize Rabbits to a Virulent Hog-Cholera Bacillus Culture.

Rabbit No.	Inoculated with.	1st injection.	2nd injection.	3rd injection.	Weight Nov. 18.	Result of subcutaneous injection of 0.000001 cc. of 24 hr. bouillon culture of Hog-cholera XII. Rabbit series, Nov. 18, 1919.
		1919	*1919*	*1919*	*gm.*	
1	Swine-typhus II, bouillon culture.	Oct. 4. 0.1 cc. subcutaneously.	Oct. 25. 0.01 cc. intravenously.	—	2,523	Lived.
2	Swine-typhus IV, bouillon culture.	Oct. 4. 0.1 cc. subcutaneously.	Oct. 25. 0.01 cc. intravenously.	—	2,700	"
3	Calf-typhus I, bouillon culture.	Oct. 4. 0.1 cc. subcutaneously.	Oct. 25. 0.01 cc. intravenously.	—	2,164	"
4	Calf-typhus III, bouillon culture.	Oct. 4. 0.1 cc. subcutaneously.	Oct. 25. 0.01 cc. intravenously.	—	2,684	"
5	*B. schottmülleri* 232, bouillon culture.	Oct. 4. 0.1 cc. subcutaneously.	Oct. 25. 0.01 cc. intravenously.	—	2,509	Death in 7 days.
6	*B. schottmülleri*, 242, bouillon culture.	Oct. 4. 0.1 cc. subcutaneously.	Oct. 25. 0.01 cc. intravenously.	—	2,561	" " 10 "
7 Control.	Calf-typhus III, suspension of agar growth, heated 1 hr. at 70°.	Oct. 17. 0.5 cc. subcutaneously.	Oct. 24. 1 cc. subcutaneously.	Nov. 1. 2 cc. subcutaneously.	2,520	" " 9 "
8 Control.	Swine-typhus II, suspension of agar growth, heated 1 hr. at 70°.	Oct. 17. 0.5 cc. subcutaneously.	Oct. 24. 1 cc. subcutaneously.	Nov. 1. 2 cc. subcutaneously.	2,752	" " 6 "
9 Control.	No previous treatment.	—	—	—	2,453	" " 8 "

The intimate relation of these organisms is shown by cross-immunization tests made with mice. These animals were immunized at the same time by subcutaneous injections of 0.0001 cc. followed by intraperitoneal injections of 0.005 cc. of 24 hour bouillon cultures. After they had recovered from the latter injection their immunity towards other cultures was tested by intraperitoneal injections of 0.005 cc. of 24 hour bouillon cultures. The results of the test are given in Table V and show that the animal and human cultures immunize against one another and that the calf culture immunized against the swine and *vice versa*.

TABLE V.

*Test of Cross-Immunity of Mice to Paratyphoids of Other Animals.**

Mice immune to.	Result of intraperitoneal injection of 0.005 cc. of 24 hr. bouillon culture.					
	Calf-typhus V.		*B. schottmülleri* 232.		Swine-typhus V.	
	Lived.	Died.	Lived.	Died.	Lived.	Died.
Calf-typhus V	4	0	4	0	4	0
B. schottmülleri 232	3	1	4	0	4	0
Swine-typhus V	3	0	3	0	3	0
Controls. No previous treatment	0	4	1	3	0	4

* See text for methods of immunization.

DISCUSSION.

The question arises whether we should place these animal paratyphoids, that culturally are the same as *Bacillus schottmülleri*, in a separate group because they fail to absorb the agglutinins from the serum of animals immune to the latter. This failure to absorb agglutinins seems to be a very fundamental difference and these organisms should be regarded as a distinct variety of paratyphoid. No differences either cultural or serological have been detected between the strains derived from swine, calves, and the few strains from the other species that have been studied.

A common name is desirable for this group of organisms and if it is found, upon further study, that these organisms are the same as the

Aertrycke bacillus, isolated by de Nobele (4) from an outbreak of food poisoning, the name of *Bacillus aertryckei* would be appropriate. It is possible that there is one host that harbors these organisms and from it the other animals become infected. If this should prove to be the case, a name indicating this host would be the logical one. On the other hand, the naming of the animal from which a particular culture is isolated is desirable and for the present I propose that they be called typhus with a prefix denoting the animal from which they were isolated. The name typhus has only its long use to commend it, as the organisms are not like *Bacillus typhosus*, nor do they produce a disease that very closely resembles typhoid fever. Another objection is that in the cultures from the smaller animals the word typhus has been used in connection with the paratyphoid disease that is so common. In my experience most of these cultures from mice and guinea pigs belong to the *enteritidis* group and can be separated by their specific agglutination characters, though organisms of the group under consideration also occur. *Bacillus enteritidis* also occurs in the larger animals. I have found it in one pig, Jensen (5), Meyer, Traum, and Roadhouse (6), and others have found it in calves with diarrhea, and Graham, Reynolds, and Hill (7) have found it in an acute disease of horses. In the future it would be well to call these *Bacillus enteritidis* and reserve the use of mouse-typhus, guinea pig-typhus, etc., for the organisms that we have considered in this paper.

It is evident that the group that has been considered in this paper has been encountered before. I have already (1) pointed out that many of the strains of so called *Bacillus suipestifer* probably belong to this group. Bock (8) noted that when he saturated the sera of animals immune to mouse-typhus, *Bacillus suipestifer*, or bacilli obtained from outbreaks of food poisoning with *Bacillus schottmülleri* the agglutinins for the last bacillus were removed while those for the first three organisms were not affected. Sobernheim and Seligmann (9) in studying paratyphoid bacilli noted that three cultures classed as *Bacillus schottmülleri* formed fine clumps in the serum of an animal immune to *Bacillus schottmülleri*. They immunized a rabbit to one of these strains heated to 70°C. and found that the serum agglutinated the three cultures to the same degree while the other *Bacillus schottmülleri* cultures were agglutinated in only the lower dilutions of serum. Especially significant

are the observations of Bainbridge and O'Brien (10). They compared cultures from cases of food poisoning with those from undoubted cases of paratyphoid fever. In *Bacillus schottmülleri* sera the former produced fine clumps and did not absorb the agglutinins for the immunizing strains, whereas the latter formed flocculent clumps and absorbed all the agglutinins from the serum. Their control cultures of *Bacillus suipestifer* acted the same as those from food poisonings, but as these controls were obtained from German laboratories it is probable that they were the same as those I have called swine-typhus. Krumwiede, Valentine, and Kohn (11) separated from *Bacillus schottmülleri* by absorption tests a number of organisms obtained from rodents which probably belong to this group.

As noted above, when suspensions of agar slant growth or killed cultures are used as antigens in agglutination tests the difference between these animal cultures and *Bacillus schottmülleri* could not be detected and it seems possible that the so called paratyphoid B bacilli that Jensen (5), Christiansen (12), and others have associated with diarrhea in calves are the same as the organisms I have called calf-typhus. Many of the paratyphoid B bacilli isolated from food poisonings quite possibly belong to the same group but from the literature one cannot draw any conclusions as the diagnosis has usually been made on the agglutination test without supplementary absorption tests. This subject is important because we want to know what type of infections man gets from the lower animals.

There are several well recognized groups of pathogenic animal paratyphoids besides *enteritidis* and the group considered here. The hog-cholera bacillus, or better, *Bacillus choleræ suis*, described by Smith (13), differs from the others by being highly pathogenic for rabbits. More recently (1) it has been shown to have distinct cultural differences from *Bacillus schottmülleri*. The Voldagsen bacillus described by Dammann and Stedefeder (14) and the "Ferkel typhus" bacillus described by Glässer (15) seem to be identical. They are differentiated from the other paratyphoids by their failure to act on mannitol and the fact that they produce little or no gas. *Bacillus abortus equi*, first described by Smith and Kilborne (16) and later studied by Meyer and Boerner (17) and others, resembles *Bacillus schottmülleri* culturally, except that it fails to produce hydrogen sulfide, and on agar forms a

dry brittle growth. Serologically Meyer and Boerner, and Murray (18) place it in a group by itself. In its virulence for rabbits Smith and Kilborne pointed out that it resembled a mildly virulent hog-cholera bacillus.

Jordan (19) and Reerstorp (20) have found a variety of so called intermediate paratyphoids in the intestinal tract of normal swine, and from children Lewis (21) and others have obtained paratyphoids which have been classified culturally by Graham-Smith (22). It is difficult to determine just what relation these paratyphoids of the normal digestive tract bear to the established groups. They differ serologically and culturally from the members of these several groups but it is conceivable that under certain conditions they might invade the body and change their characters.

Smith and Reagh (23) discussed the possibility of the host changing the agglutinative characters of organisms and such a possibility should be considered here. After passing from animal to man and becoming adapted to the latter, it is quite possible that organisms might change both their cultural and agglutinative characters. Careful study of food poisoning outbreaks due to eating meat containing these animal paratyphoids might throw some light on this subject. If such a change does occur it would result in much confusion. I have from time to time modified slightly the cultural characters of some of these paratyphoids by passage through animals, but on the whole the cultural and especially the agglutination characters are remarkably constant.

CONCLUSIONS.

1. In addition to the paratyphoid bacilli already named there exists a group which occurs in a variety of animals and which culturally is the same as *Bacillus schottmülleri*. As a rule this group can be separated from the latter by the type of clumps formed when bouillon cultures are used as antigens, while other antigens and complement fixation tests have failed to differentiate it. Agglutination absorption tests sharply separate the animal from the human paratyphoids.

2. No differences have been detected between organisms of this group derived from a number of animals and a common name for them is desirable, but for the present it seems better to call them calf-,

swine-, mouse-, etc., typhus, according to the animal from which they were isolated.

3. Evidence exists in the literature that these organisms have been associated with food infections in man, particularly with what have been called paratyphoid B infections, but this function, as well as the part they play in animal diseases, is a subject for further study.

4. Well defined groups of paratyphoid such as *Bacillus choleræ suis*, the Voldagsen bacillus, *Bacillus abortus equi*, and *Bacillus enteritidis* are found in animals in addition to the organisms considered in this paper, and every attempt should be made to range newly isolated organisms in one or the other of these well recognized groups.

5. One of the objects in continuing this work was to find a method of differentiating these animal from the human paratyphoids less complicated than agglutination absorption. This object was not realized; the two groups are very similar and agglutination absorption seems to be the only means of classifying them.

The writer is grateful to Mr. Henry Hagens of this Laboratory for valuable technical assistance.

BIBLIOGRAPHY.

1. TenBroeck, C., *J. Exp. Med.*, 1918, xxviii, 759.
2. Winslow, C.-E. A., Kligler, I. J., and Rothberg, W., *J. Bacteriol.*, 1919, iv, 429.
3. Dreyer, G., quoted by Gardner, A. D., *J. Hyg.*, 1918, xvii, 471.
4. de Nobele, J., *Ann. Soc. méd. Gand*, 1898, lxxvii, 281; 1901, lxxx, 74.
5. Jensen, C. O., in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, 2nd edition, 1913, vi, 121.
6. Meyer, K. F., Traum, J., and Roadhouse, C. L., *J. Am. Vet. Med. Assn.*, 1916, xlix, 17.
7. Graham, R., Reynolds, F. H. K., and Hill, J. F., *J. Am. Vet. Med. Assn.*, 1920, lvi, 378, 489, 586.
8. Bock, F., *Arb. k. Gsndhtsamte*, 1906, xxiv, 237.
9. Sobernheim, G., and Seligmann, E., *Z. Immunitätsforsch., Orig.*, 1910, vi, 401.
10. Bainbridge, F. A., and O'Brien, R. A., *J. Hyg.*, 1911, xi, 68.
11. Krumwiede, C., Jr., Valentine, E., and Kohn, L. A., *J. Med. Research*, 1918–19, xxxix, 449.
12. Christiansen, M., *Centr. Bakteriol., Ite Abt., Orig.*, 1917, lxxix, 196.
13. Smith, T., Hog cholera: its history, nature, and treatment, *U. S. Dept. Agric., Bureau Animal Industry*, 1889, 69.

14. Dammann, K., and Stedefeder, K., *Arch. wissensch. u. prakt. Tierheilk.*, 1910, xxxvi, 432.
15. Glässer, K., *Deutsch. tierärztl. Woch.*, 1908, No. 40, 569.
16. Smith, T., and Kilborne, F. L., *U. S. Dept. Agric., Bureau Animal Industry, Bull. 3*, 1893, 49, 53.
17. Meyer, K. F., and Boerner, F., *J. Med. Research*, 1913, xxix, 325.
18. Murray, C., *J. Infect. Dis.*, 1919, xxv, 341.
19. Jordan, E. O., *J. Infect. Dis.*, 1918, xxii, 252.
20. Reerstorp, A. R., *Den k. Vet. Landbohφiskoles Aarsskrift*, 1919, 113.
21. Lewis, C. J., *Gt. Britain Local Gov. Bd., Suppl. Rep. Med. Off.*, 1910–11, xl, 314.
22. Graham-Smith, G. S., *Gt. Britain Local Gov. Bd., Suppl. Rep. Med. Off.*, 1911–12, xli, 304.
23. Smith, T., and Reagh, A. L., *J. Med. Research*, 1903, ix, 270.

BACILLI OF THE HOG-CHOLERA GROUP (BACILLUS CHOLERÆ SUIS) IN MAN.

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(Received for publication, March 5, 1920.)

Hirschfeld (1) has described an epidemic of clinical paratyphoid fever which occurred in Serbia or Greece from which he obtained organisms culturally paratyphoid B or *Bacillus schottmülleri*, but which were not agglutinated by the sera of animals immune to the latter bacillus. These organisms were isolated eighteen times, twice being obtained after death. Sera obtained from patients in this epidemic agglutinated the organisms isolated in some cases in dilutions as high as 1:800. MacAdam (2) obtained similar inagglutinable paratyphoid bacilli in Mesopotamia from the blood stream of patients who clinically showed respiratory rather than enteric symptoms. Mackie and Bowen (3) have described cultures of the same group, as shown by Schütze's (4) work. The latter compared twelve cultures obtained from febrile cases occurring in the Balkan region, the organism isolated by the above writers being included. All the cultures were agglutinated by and absorbed the agglutinin from the serum of an animal immune to one of Hirschfeld's strains. Hirschfeld called his organisms paratyphoid C and Schütze called them Hirschfeld's bacillus.

It seemed possible that by comparing these organisms with the paratyphoids found in animals they might be classed in one of the known groups. Upon request Dr. Hirschfeld kindly sent me two cultures of these organisms and some immune serum. The cultures were labeled Para C and it may be that they were duplicates but I have called them Paratyphoid C I and II and have used both of them in the tests made. No differences have been detected between the two strains.

Agglutination tests were made with the serum received and the results given in Table I were obtained.

The serum agglutinates two strains of the hog-cholera bacillus in as high dilution as it does the organism isolated by Hirschfeld, while *Bacillus schottmülleri*, *Bacillus enteritidis*, and swine-typhus bacilli are agglutinated in only the lowest dilutions.

Anti-hog-cholera bacillus serum was absorbed with the cultures received and the results of agglutination tests with the absorbed as well as the unabsorbed serum are given in Table II.

The paratyphoid bacilli under consideration are agglutinated to the titer limit by anti-hog-cholera bacillus serum and absorb from this serum not only the agglutinins for themselves but also those for the hog-cholera bacilli.

TABLE I.

Agglutination with Hirschfeld Paratyphoid C Serum.

Culture tested.	Limit of agglutination.
B. schottmülleri 232	100
" " 242	100
Swine *enteritidis* I	50
B. enteritidis (Mt. Sinai)	50
Swine-typhus I	100
" V	100
Hog-cholera XII	12,800
" XIV	12,800
Paratyphoid C I	12,800
" " II	12,800

TABLE II.

Tests with Anti-Hog-Cholera Bacillus Serum.

Culture tested.	Limit of agglutination using serum.		
	Unabsorbed.	Absorbed with Paratyphoid C I.	Absorbed with Paratyphoid C II.
Paratyphoid C I	12,800	200—	200—
" "II	12,800	200—	200—
Hog-cholera bacillus XII	12,800	200	200
" " XVI	12,800	200	400

Rabbits were immunized, one to each of the two strains, by the injection of unheated bouillon cultures and their sera gave the results in Table III.

Four different hog-cholera bacillus cultures were agglutinated to the titer limit. Three of the hog-cholera bacillus cultures were used for absorption tests, one for one serum and two for the other, and they

took out the agglutinins for the Paratyphoid C bacilli, as well as for the other hog-cholera bacilli.

Serologically these organisms are typical hog-cholera bacilli but culturally they differ in that they form acid and gas in dulcite and arabinose and form hydrogen sulfide, whereas the hog-cholera bacillus, as has been pointed out by Jordan (5) and Krumwiede, Kohn, and Valentine (6), do not act on these carbohydrates or form hydrogen sulfide. Culturally, then, they are the same as *Bacillus schottmülleri.*

Two rabbits given subcutaneous injections of 0.1 cc. of 24 hour bouillon cultures of these organisms showed a slight rise in temperature

TABLE III.

Saturation of Paratyphoid C Sera with Hog-Cholera Bacilli.

Culture tested.	Limit of agglutination with serum of Rabbit A, immune to Paratyphoid C I.		Limit of agglutination with serum of Rabbit B, immune to Paratyphoid C II.		
	Unabsorbed.	Absorbed with Hog-cholera bacillus XVI.	Unabsorbed.	Absorbed with Hog-cholera bacillus X.	Absorbed with Hog-cholera bacillus XII.
Hog-cholera X, Pig 30	12,800	200	12,800	400	200—
" XI	12,800	200—	12,800	400	200—
" XII	12,800	200—	12,800	800	800
" XVI	12,800	200—	12,800	400	400
Paratyphoid C I	12,800	200—	12,800	400	800
" " II	12,800	200—	12,800	400	800

and local lesions. Neither was very sick and recovery was prompt. A typical hog-cholera bacillus should kill rabbits in from 6 to 10 days, so that these cultures resemble the animal typhus group rather than the hog-cholera bacilli in their virulence for rabbits.

20 days after the subcutaneous injection these rabbits were given an intravenous injection of 0.01 cc. of 24 hour bouillon cultures of the same organisms. This produced no effect and 25 days later they were given a subcutaneous injection of 0.00001 cc. of a 24 hour bouillon culture of a virulent hog-cholera bacillus. This organism in a dilution 100 times the one used here will kill normal rabbits in from 6 to 10 days, but in these injected rabbits it produced no rise in temperature, loss in weight, or local lesion. The rabbits were

killed 6 weeks after the final injection and aside from a slight enlargement of the spleen appeared normal. Cultures from the spleen showed hog-cholera bacilli from one rabbit but not from the other.

The interpretation of this experiment is not clear. As has been pointed out in the preceding paper (7), various animal typhus cultures will immunize rabbits to this virulent hog-cholera bacillus, but no tests have been made with the amount used here. The fact that these rabbits withstood such a large injection without showing any evident disturbance seems to indicate that they had a specific immunity.

A pig weighing 30 pounds was fed 100 cc. of one of the cultures received from Dr. Hirschfeld mixed with its food. For the next 4 days its temperature was increased and it ate very little. Its feces were diarrheal in character but the organism fed was not obtained on Endo plates made from the feces the 5th day after the feeding. On this day its temperature approached normal, it was eating well and appeared to be much livelier than on the days following the feeding. On the 5th day after the feeding virulent hog-cholera virus was injected intramuscularly, as it has been found in similar experiments that hog-cholera bacilli introduced into the digestive tract by feeding will act as secondary invaders when the pig is infected with virus. The pig died 8 days after the injection of the virus and was autopsied soon after death. It showed lesions characteristic of hog-cholera with the addition of a grayish, membranous necrosis of the mucosa of the large intestine. Plate cultures were made from the spleen, two mesenteric lymph nodes, the exudate in the colon, the mucous membrane of the colon after removing the exudate, the kidney, and the liver. In all, thirty-five subcultures from these plates were studied. They resembled the organism fed, with the following exceptions. Of six cultures from the mucosa of the colon, one failed to form hydrogen sulfide or ferment dulcite, but acted on arabinose. The other cultures were the same as the culture fed. Of two cultures from the kidney, one was the same as the organism fed, while the other differed in that it failed to act on dulcite. It did, however, produce hydrogen sulfide and on the second test fermented dulcite promptly. Only two cultures from the liver were examined and neither acted on dulcite, while one did and the other did not ferment

arabinose. Both formed hydrogen sulfide and on the second test both fermented dulcite and arabinose. The culture from the colon is, then, the only one that has shown any permanent change, and this culture 2 months after its isolation still failed to form hydrogen sulfide or ferment dulcite. It has, then, approached the hog-cholera bacillus in its cultural characters. The organisms from the liver and kidney also approached the hog-cholera bacillus in cultural character, but they soon regained the properties lost. The question as to whether the cultures recovered are the ones fed cannot positively be decided. Seven other pigs of the same litter have been infected with hog-cholera virus and bacteriological examination has failed to show the hog-cholera bacillus, though from some of them swine-typhus bacilli have been isolated. All the cultures from this pig resembled the hog-cholera bacillus serologically so the probabilities are that they were the descendants of organisms fed.

One similar test has been made with a swine-typhus culture and in this one case the feeding failed to cause a rise in temperature. Cultures made at autopsy, the animal having been infected with hog-cholera virus, failed to show swine-typhus bacilli.

When hog-cholera bacilli are fed, the pig reacts as did the animal fed the Paratyphoid C culture. There is an increased temperature beginning the day after the feeding and lasting for from 3 to 4 days. If at the end of this time hog-cholera virus is injected into the pig, hog-cholera bacilli will be found in the organs at autopsy.

DISCUSSION.

While these organisms isolated by Hirschfeld (1) are not typical hog-cholera bacilli in that they ferment dulcite and arabinose, produce hydrogen sulfide, and are not virulent for rabbits, their serum reactions are so characteristic that they should be placed in the hog-cholera bacillus group. These serum reactions are very fundamental, much more so than are the fermentations of the rarer carbohydrates.

There are several possible explanations which might account for these differences. One is that in the region from which these organisms were obtained atypical strains of hog-cholera bacilli exist in swine. Another is that the organisms in swine may be typical but

after passing to man they have become modified. A third possibility is that these organisms did not come from swine. They do not, however, correspond to any of the animal paratyphoids that have been described. What appear to be culturally typical hog-cholera bacilli do exist in nearby regions, as is shown by the observations of Trawinski (8). He isolated forty-two cultures from swine imported into Germany from Poland. All the cultures failed to ferment dulcite and arabinose, while three cultures of so called *Bacillus suipestifer* obtained from Kral's collection acted on these carbohydrates. One so called *suipestifer* strain obtained from Budapest acted the same as the cultures he isolated and was agglutinated to the titer limit by serum of an animal immune to one of his strains. The cultures from Kral were not agglutinated to the titer limit by this serum. He does not record the virulence of the cultures for rabbits or the production of hydrogen sulfide, but notes that all of his forty-two cultures formed acid but no gas in sorbite, whereas the control cultures of *Bacillus suipestifer* formed gas.

It is an interesting fact that the hog-cholera bacillus, which at one time was so commonly present in swine infected with hog-cholera that it was regarded as the cause of the disease, has not been found more frequently in man. In the older literature of food poisonings some of the organisms isolated were virulent when injected subcutaneously into rabbits and in some cases necroses were found in the livers of these animals. These facts indicate that the hog-cholera bacillus may have been the organism that was being studied, but the evidence is not conclusive.

Reed and Carroll (9) made a comparative study of *Bacillus icteroides* (Sanarelli) and the hog-cholera bacillus and concluded that they were the same. In cultural characters, virulence, and the disease produced in animals the two cultures were identical. They made only a few agglutination tests and the results are not very clear-cut but they indicate a relation between the two organisms. We are fortunate in having in our collection a culture of *Bacillus icteroides* that was received directly from Sanarelli and it is agglutinated to the titer limit in anti-hog-cholera bacillus serum and absorbs the agglutinins from this serum. In addition it fails to ferment dulcite and arabinose and does not produce hydrogen sulfide, thus resembling the hog-

cholera bacillus, while it differs from the latter organism in that it is not virulent for rabbits.

There is another culture in the collection labeled paratyphoid B Longcope, which culturally and serologically is a hog-cholera bacillus but which is not virulent for rabbits. The chances are that this culture came from a case of paratyphoid reported by Longcope (10) in 1902, but we cannot be sure of this fact. The probabilities are that it is at least of human origin.

As far as I know these are the only cultures from man that correspond closely to the hog-cholera bacillus so that an outbreak in the Balkan region with which organisms of the hog-cholera bacillus group are associated is of great interest. There must be many opportunities for hog-cholera bacilli to infect man, but they either rarely find conditions such that they can grow in the human body or, what is less likely, they do grow and quickly lose their distinguishing characters.

CONCLUSIONS.

1. The organisms isolated by Hirschfeld from febrile cases resembling paratyphoid fever and named Paratyphoid C can be placed in the hog-cholera bacillus group by their agglutination absorption properties though they are not typical culturally.

2. When fed to a pig a febrile disease resulted from which the animal recovered. After injection of hog-cholera virus the organisms fed were found generally distributed and some of them had lost cultural characters so that they are brought into the class of typical hog-cholera bacilli except for their low virulence for rabbits.

3. While hog-cholera bacilli have many opportunities to infect man they either are not able to grow in the human body or, what is less likely, they do grow and lose the characters that distinguish them.

The writer is indebted to Dr. L. Hirschfeld, Director of the Laboratory Service of the Serbian Army, for the cultures, to Professor David Klein, of the School of Hygiene, the Johns Hopkins University, for bringing them to this country, and to Mr. Henry Hagens, of this Laboratory, for technical assistance.

BIBLIOGRAPHY.

1. Hirschfeld, L., *Lancet*, 1919, i, 296.
2. MacAdam, W., *Lancet*, 1919, ii, 189.
3. Mackie, F. P., and Bowen, G. J., *J. Roy. Army Med. Corps*, 1919, xxxiii, 154.
4. Schütze, H., *Lancet*, 1920, i, 93.
5. Jordan, E. O., *J. Infect. Dis.*, 1917, xx, 457.
6. Krumwiede, C., Jr., Kohn, L. A., and Valentine, E., *J. Med. Research*, 1918, xxxviii, 89.
7. TenBroeck, C., *J. Exp. Med.*, 1920, xxxii, 19.
8. Trawinski, A., *Centr. Bakteriol., 1te Abt., Orig.*, 1918, lxxx, 339.
9. Reed, W., and Carroll, J., *J. Exp. Med.*, 1900, v, 215.
10. Longcope, W. T., *Am. J. Med. Sc.*, 1902, cxxiv, 209.

STUDIES ON CALCIUM AND MAGNESIUM METABOLISM IN DISEASE.

I. Calcium and Magnesium Metabolism in Leprosy.

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(Received for publication, February 16, 1920.)

In a preliminary paper by Honeij[1] on bone changes in leprosy it was shown that in this disease there is a definite absorption of bone salts. The idea seems prevalent that the bony changes in leprosy are due to suppurative processes or to nerve lesions, and hence little attention has been paid to them. This view-point is, however, entirely erroneous, for although bony changes may bear a relation to suppurative processes and to nerve lesions, Honeij points out that this kind of reaction plays no part in the true type of bone absorption which is part of the general disease. Thus, in the cases of leprosy with absence of suppuration and nerve disturbances the absorption of bone salts is demonstrable. Again, in progressive cases without any marked evidence of nerve involvement and distinctly without indication of suppuration the absorption of bone salts and consequent changes are most noticeable.

From the foregoing considerations it is apparent that a study of the calcium and magnesium exchange in leprosy would possibly afford a better conception of the processes involved in the abnormal condition under discussion. Indeed, the possibility exists that such a study might lead to improvement in treatment or prognosis as well as be of importance in the classification of the various types of leprosy.

[1] Honeij, J. A., *Am. J. Roentgenol.*, 1917, iv, N. S., 494.

Plan of Investigation.

Experience has demonstrated that simple determination of the balance of calcium and magnesium in the body does not always lead to convincing conclusions. This is due in large measure to the fact that it is difficult to obtain approximately perfect balances, and even though attained this maintenance is not easy. An attempt to draw definite conclusions from the balances alone is even more difficult in diseased conditions wherein the elements concerned exert a disturbing influence.

Therefore in the present investigation, although due weight has been given to the calcium and magnesium balances, more significance has been attached to giving the diseased organism a distinct task to perform and observing the manner in which this is accomplished. Stated differently, the general plan of procedure has been to maintain diseased individuals upon a restricted diet low in calcium and magnesium and then for a period to increase the calcium or magnesium either by administration of food rich in the element or directly as a salt. From the data so obtained various ratios were calculated. A comparison was then made with the ratios obtained from normal individuals maintained under similar experimental conditions. In this manner deviations from normal behavior are readily detected, inasmuch as usually, when perverted calcium or magnesium exchange occurs, the evidence is quite noticeable.

Method.—The dietary was limited in the kind of food, and, as has been stated previously, was so selected as to be poor in calcium and magnesium quantitatively. The dietary was restricted within certain limits but was not a constant diet. Calcium was added to this diet in the form of milk, magnesium as magnesium citrate. The investigation was divided into four periods. Previous to the first period all subjects were maintained upon the diet of Period 1 for 3 days. Period 1 was arranged so as to represent a calcium-magnesium-low interval. Period 2 represented a calcium-rich but magnesium-low period. This was accomplished by the addition of varying quantities of milk. Period 3 was approximately identical with Period 1; that is, a period low in both calcium and magnesium, milk being withdrawn from the dietary. Period 4 was essentially the same as Period 3 except that 2 gm. of magnesium citrate, equivalent to 205 mg. of magnesium, were admin-

istered in three equal doses daily. In other words, Period 4 was an interval of low calcium and high magnesium.

The experimental periods were marked off in the feces by the employment of carmine. Calcium and magnesium were determined in the food, urine, and feces by the methods of McCrudden.[2]

Description of Subjects.

Normal Individuals.

Subject 1.—M. A., female, age 25 years; engaged in laboratory work. The experiment was so planned that menstrual periods were avoided.

Subject 2.—G. S., male, age 28 years; engaged in laboratory work.

Leprous Cases.

Case 1.—M. T., male, age 31 years; single; laborer; born in Greece. December 1, 1916. Admitted to the New Haven Hospital.

Complaint.—"Red spots on body." A few spots first appeared on the face, resembling lesion now present. These lesions were at first small and red, later became browner and larger. Cheeks were at first affected, then the feet, following these the arms and hands. Lesions are not painful, have never itched, never broken down to form ulcers. Patient claims he feels well. For less than a year the left side of the nose has been almost completely blocked; no catarrh previously.

Family History.—Negative.

Past History.—Negative.

Physical Examination.—Negative except in the following respects. The nose shows thickening of the septum on the left side, with whitish color and several small whitish nodules. Glands of the cervical, axillary, epitrochlear, and inguinal regions are all moderately enlarged. The skin shows the greatest changes. On the face and forehead rather numerous, flat papules 2 to 4 mm. in size and of a brownish color. An infiltrated area 1 cm. in diameter seen on right cheek and two similar areas on left cheek. Pigmented areas also seen. A nodule 1.5 cm. in diameter on left forehead, involving skin and subcutaneous tissue. The same lesions are found on forearm, elbows, buttocks, thighs, and lower legs, mostly on extensor surfaces. The nerves are thickened; the ulnar nerves, peroneal, and popliteal are also affected. Many large papules and a large area on left shin are anesthetic. Many pigmented areas are slightly affected. The blood examination on November 8, 1917, shows 63 per cent hemoglobin and 8.4 per cent white cells, mostly polymorphonuclears. Wassermann reaction +++. Later the white cell blood count was 11,000 with 75 per cent polymorphonuclears. Achroma also.

[2] McCrudden, F. M., *J. Biol. Chem.*, 1909–10, vii, 83; 1911–12, x, 187.

Renal functional test, December 18, 1917, shows 54 per cent excretion 2 hours after phenolsulfonephthalein. The urine examination gave a specific gravity of 1,015; the color was amber; clear; acid reaction; slight possible trace of albumin; no sugar; few epithelial cells on microscopic examination.

The radiographic gastrointestinal examination was negative except for some colonic stasis.

The patient was under treatment for 1 year and on special diet from June 26 to July 25, 1917.

Diagnosis.—Leprosy of the nodular, anesthetic type.

Case 2.—G. C., male, age 21 years; single; laborer; born in Italy. January 2, 1918. Admitted to the New Haven Hospital.

Complaint.—Difficulty in breathing through nose. Patient has been troubled with a dry feeling in his nose for the last 2 years. Has expelled by force scab-like masses which are now and then accompanied by slight epistaxis. Has always had a biting sensation in his nose. Patient is not subject to colds. Throat troubles him also. Headaches are frequent and quite frequently has cold sweats. Every now and then his upper extremity "falls asleep," giving tingling sensation. Itchy feeling when body becomes warm.

Family History.—Negative.

Past History.—Negative.

Physical Examination.—Negative except in the following respects. The nose shows both anterior nares almost entirely occluded by atrophic scabs. On removing them a perforation of the septum is seen 3 by 2 cm.; it does not seem to include the bony portion of the septum. The turbinates are difficult to determine. The eyes show a slight conjunctivitis and areas of anesthesia which also occur on lids. A small nodule is seen at the sclera-corneal margin of both eyes. The glands of the cervical, axillary, and inguinal regions are enlarged. The examination of the body is negative except for areas of anesthesia, fairly symmetrical in their distribution, occurring on body and on extremities, face, and ears. The hands and feet are cyanotic. The patient perspires freely. The nerves are thickened. The ulnar and anterior tibial nerves are considerably thickened. Areas of discoloration and pigmentation seen on the buttocks; here there are also some nodules. The blood examination on October 16, 1918, shows a white blood count of 8,400 and on October 21 a count of 13,600. The urine examination on October 15 gave a specific gravity of 1,032; normal color; clear; acid reaction. Microscopic examination showed a rare leucocyte.

Diagnosis.—Leprosy of the anesthetic type. An earlier case of the disease than Case 1.

Dietary.

The diet for all subjects consisted of varying but definite quantities of the foodstuffs presented in Table I. The calcium content and magnesium content are also given. In order to save space the quantities of food consumed daily are omitted.

TABLE I.

Dietary.

Food.	Calcium content.	Magnesium content.
	per cent	*per cent*
Bread	0.032	0.036
Orange	0.056	0.015
Egg	0.073	0.015
Sugar		
Baked potato	0.005	0.020
Chopped beef	0.007	0.018
Banana	0.013	0.021
Prunes	0.042	0.018
Boiled rice	0.002	0.005
Fresh strawberries	0.041	0.019
Milk	0.179 0.199	0.016 0.014

Calcium and Magnesium Balances.

In Tables II to V are presented the data relative to the calcium and magnesium exchange in the four subjects of investigation. The first period shows the two normal subjects to be in a distinct negative calcium balance, but on the other hand, the magnesium balance is positive. Added calcium, in the form of milk, in the second period causes both subjects to approach an equilibrium. In Subject 1 the added calcium exerted little or no influence upon the magnesium balance, whereas with the other normal individual, Subject 2, a distinct negative magnesium balance was exhibited. Withdrawal of the additional calcium in the third period resulted with the two normal subjects in a distinct negative calcium balance; the negative magnesium balance in this period was not so marked. In the fourth period where magnesium citrate was added to the diet a significant

TABLE II.

Subject 1; Normal. Calcium and Magnesium Balances.

Period.	Diet.	Date.	Calcium. Intake.	Calcium. Output. Urine.	Calcium. Output. Feces.	Calcium. Balance.	Magnesium. Intake.	Magnesium. Output. Urine.	Magnesium. Output. Feces.	Magnesium. Balance.
		1919	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1	Calcium-low and magnesium-low.	Jan. 4	317	316			228	67		
		" 5	349	300			239	93		
		" 6	333	335			263	96		
		" 7	342	395			275	88		
Total			1,341	1,346	885	−890	1,005	344	585	+76
				2,231				929		
Average per day			335	336	221	−222	251	86	146	+19
				557				232		
2	Calcium-high and magnesium-low.	Jan. 8	1,324	469			341	109		
		" 9	1,333	508			361	110		
		" 10	1,339	618			392	134		
Total			3,996	1,595	2,500	−99	1,094	353	676	+65
				4,095				1,029		
Average per day			1,332	532	833	−33	364	117	225	+22
				1,365				343		
3	Calcium-low and magnesium-low.	Jan. 11	365	325			294	108		
		" 12	329	352			277	124		
		" 13	358	343			271	114		
Total			1,052	1,020	881	−849	842	346	691	−195
				1,901				1,037		
Average per day			351	340	293	−283	280	115	230	−65
				633				345		
4	Calcium-low and magnesium-high.	Jan. 14	367	351			487	128		
		" 15	419	426			507	152		
		" 16	408	408			533	148		
Total			1,194	1,185	1,031	−1,022	1,527	428	710	+389
				2,216				1,138		
Average per day			398	395	343	−341	509	142	236	+130
				738				379		

TABLE III.

Subject 2; Normal. Calcium and Magnesium Balances.

Period.	Diet.	Date.	Calcium. Intake.	Calcium. Output. Urine.	Calcium. Output. Feces.	Calcium. Balance.	Magnesium. Intake.	Magnesium. Output. Urine.	Magnesium. Output. Feces.	Magnesium. Balance.
		1919	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1	Calcium-low and magnesium-low.	Jan. 4	506	149			345	128		
		" 5	542	215			398	124		
		" 6	537	213			369	112		
		" 7	584	242			429	104		
		" 8	525	359			377	161		
Total			2,694	1,178	2,264	−748	1,918	629	1,257	+32
				3,442				1,886		
Average per day			539	235	452	−150	383	125	251	+6
				688				377		
2	Calcium-high and magnesium-low.	Jan. 9	1,539	295			494	176		
		" 10	1,467	304			450	183		
		" 11	1,499	400			489	165		
Total			4,505	999	3,479	+27	1,433	524	1,132	−223
				4,478				1,656		
Average per day			1,501	333	1,159	+9	477	174	377	−74
				1,492				552		
3	Calcium-low and magnesium-low.	Jan. 12	444	207			363	134		
		" 13	502	215			382	145		
		" 14	537	415			417	110		
Total			1,483	837	1,735	−1,089	1,162	389	822	−49
				2,572				1,211		
Average per day			494	279	578	−363	387	129	274	−16
				857				403		
4	Calcium-low and magnesium-high.	Jan. 15	544	280			612	185		
		" 16	472	382			560	169		
		" 17	592	772			595	101		
Total			1,608	1,434	1,390	−1,216	1,767	455	976	+336
				2,824				1,431		
Average per day			536	478	463	−405	589	151	325	+112
				941				477		

TABLE IV.

Case 1; Leprosy. Calcium and Magnesium Balances.

Period.	Diet.	Date.	Calcium. Intake.	Calcium. Output. Urine.	Calcium. Output. Feces.	Calcium. Balance.	Magnesium. Intake.	Magnesium. Output. Urine.	Magnesium. Output. Feces.	Magnesium. Balance.
		1917	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1	Calcium-low and magnesium-low.	June 30	290	22			194	60		
		July 1	217	21			174	77		
		" 2	277	23			198	77		
		" 3	299	23			184	69		
Total			1,083	89	560	+434	750	283	223	+244
				649				506		
Average per day			271	22	140	+108	187	71	56	+61
				162				126		
2	Calcium-high and magnesium-low.	July 4	894	23			204	89		
		" 5	1,005	20			214	81		
		" 6	976	25			226	100		
Total			2,875	68	1,083	+1,724	644	270	230	+144
				1,151				500		
Average per day			958	22	361	+574	214	90	76	+48
				383				166		
3	Calcium-low and magnesium-low.	July 7	203	12			126	63		
		" 8	265	28			171	75		
		" 9	177	25			127	77		
Total			645	65	931	−351	424	215	299	−90
				996				514		
Average per day			215	22	310	−117	141	71	99	−30
				332				171		
4	Calcium-low and magnesium-high.	July 10	286	27			408	94		
		" 11	253	27			362	95		
		" 12	257	28			395	92		
Total			796	82	761	−47	1,165	281	490	+394
				843				771		
Average per day			265	27	253	−15	388	93	163	+131
				281				257		

TABLE V.

Case 2; Leprosy. Calcium and Magnesium Balances.

Period.	Diet.	Date.	Calcium. Intake.	Calcium. Output. Urine.	Calcium. Output. Feces.	Calcium. Output.	Calcium. Balance.	Magnesium. Intake.	Magnesium. Output. Urine.	Magnesium. Output. Feces.	Magnesium. Output.	Magnesium. Balance.
		1917	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1	Calcium-low and magnesium-low.	Feb. 10	523	103				324	114			
		" 11	522	129				344	139			
		" 12	486	153				339	106			
		" 13	496	119				331	87			
Total			2,027	504	1,538	2,042	−15	1,338	446	994	1,440	−102
Average per day			506	126	384	510	−4	334	111	248	360	−26
2	Calcium-high and magnesium-low.	Feb. 14	1,519	207				381	122			
		" 15	1,466	179				362	126			
		" 16	1,929	189				448	126			
Total			4,914	575	2,944	3,519	+395	1,191	374	981	1,355	−164
Average per day			1,638	192	981	1,173	+131	397	124	327	451	−54
3	Calcium-low and magnesium-low.	Feb. 17	492	135				339	109			
		" 18	517	159				353	124			
		" 19	515	161				379	115			
Total			1,524	455	1,169	1,624	−100	1,071	348	852	1,200	−129
Average per day			508	151	389	541	−33	357	116	284	400	−43
4	Calcium-low and magnesium high.	Feb. 20	562	145				592	144			
		" 21	514	152				580	148			
		" 22	525	138				542	151			
Total			1,601	435	1,137	1,572	+29	1,714	443	1,122	1,565	+149
Average per day			533	145	379	524	+9	571	147	374	521	+49

negative calcium balance persisted, but the magnesium balance became positive.

It is therefore evident that with normal subjects in negative calcium balance added calcium causes the balance to become positive and that the additional calcium exerts little or no influence upon the magnesium balance. The influence of the added calcium is, however, only temporary, since its withdrawal results in a resumption of a significant negative calcium balance. Added magnesium changes a slightly negative magnesium balance to a strongly positive one without a very significant influence upon the calcium balance.

With the leprosy cases a different picture is presented. Although both subjects were maintained on a low calcium intake in Period 1, Case 1 was in positive calcium balance and Case 2 was almost in perfect equilibrium. Case 1 was also in positive magnesium balance, whereas Case 2 showed a negative balance. Administration of added calcium in Period 2 demonstrated in both instances retention of calcium, greater in Case 1 than in Case 2. With Case 1 the magnesium balance was little altered, whereas with Case 2 the balance was more strongly negative than it was in Period 1. In Period 3, during which the additional calcium was eliminated, both calcium and magnesium were negative with respect to equilibrium. In the fourth period added magnesium caused a positive magnesium balance, and with Case 2 a positive calcium balance was also obtained. With Case 1 an approach to calcium equilibrium was evident.

A comparison of these results with those of the normal subjects shows that there is an apparent tendency for the leprosy subjects to remain in positive balance with respect to calcium, and in Case 1 in magnesium balance also. It is also evident that this tendency is, with respect to calcium, greater in the more advanced case, No. 1, than in the other patient. It is therefore indicated that in leprosy the organism exhibits a tendency to retain calcium whether maintained upon a diet containing little or much of this element, the more advanced the pathological condition clinically the more evident does this tendency to retention become.

In leprosy the organism may or may not retain a large portion of the added magnesium. Where retention was in evidence its degree was not strikingly greater than in the normal individuals maintained under similar conditions.

Further Analysis of the Data.

Calcium Metabolism.

In order to ascertain the correctness of the statement that in leprosy there is a tendency to retain calcium, the data have been subjected to further analysis.

Excretion of Added Calcium.—If one calculates the percentage of added calcium eliminated from the body during the period of administration, *i.e.* Period 2, it will be found that in leprosy the figures are different from those for normal individuals (Table VI).

TABLE VI.

Excretion of Added Calcium.

Subject.	Excretion of added calcium.*
	per cent
Subject 1; normal	100+
" 2; "	92+
Case 1; leper	38+
" 2; "	62+

* Period 2; calcium added as milk.

From these figures it is apparent that normal individuals in negative calcium balance promptly eliminate added calcium almost quantitatively, only a small amount of added calcium being retained; this leads to a corresponding approach to calcium equilibrium. In leprosy added calcium is retained in large measure. In the two individuals studied a marked difference was observed in the ability of the body to retain calcium. The query is pertinent whether this difference in behavior is due to a difference in the stage of the disease. Clinically the two cases are distinct, Case 1 being in a more advanced stage than Case 2. From the fact that the more advanced case shows the greater retention of calcium it may be argued that this subject has the greater need for this element.

Relation of Calcium Output to Intake.—From an inspection of Table VII it is apparent that in leprosy calcium exchange is different from that found in normal individuals. Placed upon a low calcium

diet the leprous subjects exhibited a distinct tendency to retain calcium, the retention being especially marked in Case 1, the more advanced case. When placed upon a calcium-rich diet Case 1 retained enormous quantities of calcium, the other subject, Case 2, exhibiting this behavior to a much less marked degree. When again placed upon a calcium-poor diet both subjects tended to approach the normal individuals in their behavior. On the other hand, the

TABLE VII.

Relation of Calcium Output to Intake.

Ratio: $\frac{\text{Calcium Output}}{\text{Calcium Intake}}$.

Subject.	Period.			
	1	2	3	4
Subject 1; normal	1.6	1.0	1.8	1.9
" 2; "	1.2	0.9	1.7	1.7
Case 1; leper	0.6	0.4	1.5	1.0
" 2; "	1.0	0.7	1.0	0.9

excretion was less marked again in the fourth period, and evidence of a tendency to retention is to be observed. This approach to a normal type of excretion may perhaps be interpreted to mean that the leprous organism once having a sufficiency of calcium for its present needs rather slowly parts with the excess.

Relation of Output to Intake for the Entire Period.—Comparison (Table VIII) of the total output in relation to intake for the entire

TABLE VIII.

Relation of Calcium Output to Intake for the Entire Period.

Subject.	Calcium.		
	Output.	Intake.	Difference.
	mg.	*mg.*	*mg.*
Subject 1; normal	10,443	7,583	−2,860
" 2; "	12,626 (13 days).	9,752	−2,874
Case 1; leper	3,639	5,399	+1,760
" 2; "	8,757	10,066	+1,309

period of investigation in the four subjects brings out the fact already alluded to; namely, a significant calcium retention in leprosy, even though the subjects were maintained for three periods out of four on a calcium-low diet. Again the fact is emphasized that in the more advanced patient, Case 1, the retention is greater than in the less advanced patient, Case 2. The normal subjects, on the other hand, exhibited a marked loss of calcium.

Relation of Calcium Intake and Output to Body Weight.—The weights of the individuals under experimentation varied widely and it is possible that this variation may bear a relation to the interpretation of the facts discussed above. With this idea in mind the relation of the total intake and output of calcium to body weight

TABLE IX.

Relation of Calcium Intake and Output to Body Weight.

Subject.	Body weight.	Calcium.		Calcium per pound of body weight.	
		Intake.	Output.	Intake.	Output.
	lbs.	*mg.*	*mg.*	*mg.*	*mg.*
Subject 1; normal.....	105	7,583	10,443	72	99
" 2; "	150 (13 days).	9,752	12,626	65	84
Case 1; leper.....	117	5,399	3,639	46	31
" 2; "	147	10,066	8,757	68	59

has been expressed numerically in Table IX. Inspection of these data will show at once the same general type of differences already discussed, so that it may be concluded that variations in body weight do not account for the fact that in leprosy calcium is used differently than in the normal organism.

Absorption of Calcium.—A point of considerable interest in the interpretation of the data presented relates to the question of variation in absorption of the introduced calcium. It is obvious that this question does not lend itself readily to dogmatic statements, since it is probable that calcium is excreted in variable degree by both the kidney and intestine under diverse circumstances. However, in the present discussion it is pertinent to compare the absorption of calcium in leprosy with that of normal individuals. In this

consideration the excretion of calcium through the urine is taken tentatively as evidence of absorption; the passage of calcium from the body through the feces as unabsorbed calcium. This is obviously incorrect, since undoubtedly part of the calcium eliminated with the feces is calcium which underwent absorption and is merely excreted by the intestine. For a comparative study of absorption, however, such a hypothesis may be employed.

On the basis of this hypothesis, with the necessary limitations involved, a study of the excretion of calcium by way of the urine in relation to the intake shows some noteworthy features (Table X). With the normal individuals on a calcium-low diet from approximately one-half to a quantity equal to the ingested calcium is excreted

TABLE X.

Relation of Urinary Calcium Excretion to Calcium Intake.

Ratio: $\frac{\textit{Calcium of Urine}}{\textit{Calcium of Food}}$.

Subject.	Period.			
	1	2	3	4
Subject 1; normal	1.0	0.4	1.0	1.0
" 2; "	0.4	0.2	0.5	0.8
Case 1; leper	0.08	0.02	0.1	0.1
" 2; "	0.2	0.1	0.3	0.2

by way of the kidney. As a calcium-rich diet is introduced the relative quantity of food calcium absorbed diminishes to a perceptible degree. Withdrawal of the calcium-rich diet causes the relation between the calcium excreted by the kidney and that ingested to resume the status which obtained in the calcium-poor period previously.

With the cases of leprosy the ratio is radically different from that of the normal individuals. In both cases relatively little calcium is eliminated by way of the urine. This is especially noticeable with the more advanced patient, Case 1. On a calcium-rich diet both subjects follow the general type of behavior of normal subjects; the degree of deviation is markedly different. The same fact holds when the calcium-poor diet is again introduced.

Relation of Fecal Calcium to Calcium Intake.—The figures given, when applied to the hypothesis outlined above, would lead one to conclude that very much less calcium was absorbed in the leprous cases than in the normal individuals. However, before positive conclusions can be drawn relative to this point a study should be made of the relation of calcium excretion by way of the feces to the calcium intake. This has been done and the results are expressed in Table XI. These figures demonstrate that normal conditions exist with respect to calcium elimination by the intestine in leprosy when these subjects are placed upon a calcium-poor diet. Addition of calcium to the diet results in a relatively smaller quantity

TABLE XI.

Relation of Fecal Calcium to Calcium Intake.

Ratio: $\frac{\textit{Calcium of Feces}}{\textit{Calcium of Food}}$.

Subject.	Period.			
	1	2	3	4
Subject 1; normal	0.6	0.6	0.8	0.8
" 2; "	0.8	0.7	1.1	0.8
Case 1; leper	0.5	0.3	1.4	0.9
" 2; "	0.7	0.6	0.7	0.7

appearing in the feces during this period. Since in this period there is no increase in either the urinary or fecal excretion of calcium it is obvious that the added calcium was absorbed in large measure. This theory is supported by the fact that in the later periods upon a calcium-poor diet some of the retained calcium is excreted by both the urine and feces (see Periods 3 and 4, Tables X and XI). The conclusion is therefore warranted that the behavior of leprous subjects with respect to urinary calcium excretion cannot be ascribed to lack of absorption of this element. Moreover, absorption of calcium in the leprous condition seems even better than in the normal individual. This is especially true with the more advanced patient, Case 1.

Relation of Urinary Calcium to Fecal Calcium.—If a comparison is made of the output of calcium in the urine and feces it becomes evident (Table XII) that only a small proportion of absorbed calcium is excreted in the more advanced case of leprosy. With the less advanced case the behavior is more nearly normal.

TABLE XII.

Relation of Urinary and Fecal Calcium.

Ratio: $\frac{\text{Calcium of Urine}}{\text{Calcium of Feces}}$.

Subject.	Period.			
	1	2	3	4
Subject 1; normal	1.5	0.6	1.1	1.1
" 2; "	0.5	0.2	0.4	1.0
Case 1; leper	0.1	0.06	0.07	0.1
" 2; "	0.3	0.2	0.4	0.3

When compared to normal individuals under the same experimental conditions leprous patients retain calcium in relatively large quantities whether the individual is maintained upon a calcium-poor or a calcium-rich diet, and the greater the intake the greater is the relative retention. In the more advanced stage of the disease the degree of retention is greater than in the early phase. This retention cannot be explained by differences of calcium intake, or by variation in the power of absorption. It is apparently an evidence of metabolic demand for calcium induced by the disease. Clinically, it may be inferred that there is a need for calcium; experimentally, the organism gives demonstration of this need by retention of calcium administered.

It is perhaps self-evident that the loss of bone salts in leprosy is but a manifestation of the disease processes. Nevertheless, it is conceivable that under dietary conditions in which calcium is not particularly abundant the lack of this element may play a material part in the rapidity of the progress of the disease. Conversely, the suggestion is pertinent that the progress of the disease may be greatly retarded or perhaps even alleviated if an abundance of calcium is

present in the diet. The suggestion that plenty of calcium should be supplied in the food as a therapeutic measure is at least worthy of trial.

Magnesium Metabolism.

Excretion of Added Magnesium.—From the figures in Table XIII it is apparent that normal individuals in a slightly negative magnesium balance retain more than one-half the magnesium added to a diet relatively low in this element. Of the leprosy patients, Case 1 behaved in a manner very similar to the normal individuals, whereas in the less advanced patient, Case 2, three-quarters of the administered magnesium was promptly eliminated.

TABLE XIII.

Excretion of Added Magnesium.

Subject.	Excretion of added magnesium.
	per cent
Subject 1; normal	36
" 2; "	45
Case 1; leper	36
" 2; "	75

Relation of Magnesium Output to Intake.—As may be seen from an inspection of Table XIV leprous individuals differ little from normal individuals in the manner in which magnesium is eliminated from the body, whether these subjects are maintained upon a diet low or rich in magnesium.

TABLE XIV.

Relation of Magnesium Output to Intake.

$$\text{Ratio: } \frac{\text{Magnesium Output}}{\text{Magnesium Intake}}.$$

Subject.	Period.			
	1	2	3	4
Subject 1; normal	0.9	0.9	1.2	0.7
" 2; "	0.9	1.1	1.0	0.8
Case 1; leper	0.6	0.8	1.2	0.7
" 2; "	1.0	1.1	1.1	0.9

Relation of Output to Intake for the Entire Period.—It is only when comparison is made between the total output and total intake of magnesium that a marked difference can be observed between normal individuals and leprous subjects. From Table XV it is seen that in spite of the fact that Case 1 ingested only about one-half or less of the magnesium taken in by the normal subjects, yet the absolute quantity of magnesium retained was more than double that of the normal subject retaining the most. It would appear, therefore, that in this case there is indication of a distinct magnesium retention. This would naturally be expected, since clinically in this patient there was evidence of considerable bone absorption. In Case 2, however, evidence of bone absorption was less apparent. With this

TABLE XV.

Relation of Magnesium Output to Intake for the Entire Period.

Subject.	Magnesium.		
	Output.	Intake.	Difference.
	mg.	*mg.*	*mg.*
Subject 1; normal	4,133	4,468	+335
" 2; " (13 days)	5,806	5,894	−88
Case 1; leper	2,291	2,983	+692
" 2; "	5,560	5,314	−246

subject there is a small loss of magnesium rather than a retention. It is therefore probable that in the advanced stages of leprosy the organism conserves its magnesium store, since it retains a considerable quantity. Although the advanced case of leprosy retained about the same percentage of food magnesium, the absolute amount retained was much greater, as may be seen from Table XV.

Relation of Magnesium Intake and Output to Body Weight.—When a comparison is made of the intake and output of magnesium to body weight it may be seen (Table XVI) that although the intake of the advanced leprous patient, Case 1, was much smaller than that of either the normal subjects or the other patient, nevertheless more magnesium was stored.

TABLE XVI.

Relation of Magnesium Intake and Output to Body Weight.

Subject.	Body weight.	Magnesium.		Magnesium per pound of body weight.	
		Intake.	Output.	Intake.	Output.
	lbs.	*mg.*	*mg.*	*mg.*	*mg.*
Subject 1; normal	105	4,468	4,133	42	39
" 2; "	150 (13 days).	5,894	5,806	39	38
Case 1; leper	117	2,983	2,291	25	19
" 2; "	147	5,314	5,560	36	38

Absorption of Magnesium.—From a study of Tables XVII and XVIII it is evident that the absorption of magnesium in leprosy falls within normal limits.

TABLE XVII.

Relation of Urinary Magnesium Excretion to Magnesium Intake.

$$\textit{Ratio: } \frac{\textit{Magnesium of Urine}}{\textit{Magnesium of Food}}.$$

Subject.	Period.			
	1	2	3	4
Subject 1; normal	0.3	0.3	0.4	0.2
" 2; "	0.3	0.3	0.3	0.2
Case 1; leper	0.3	0.4	0.5	0.2
" 2; "	0.3	0.3	0.3	0.2

TABLE XVIII.

Relation of Fecal Magnesium to Magnesium Intake.

$$\textit{Ratio: } \frac{\textit{Magnesium of Feces}}{\textit{Magnesium of Food}}.$$

Subject.	Period.			
	1	2	3	4
Subject 1; normal	0.5	0.6	0.8	0.4
" 2; "	0.6	0.8	0.7	0.5
Case 1; leper	0.3	0.3	0.7	0.4
" 2; "	0.7	0.8	0.8	0.6

Relation of Urinary Magnesium to Fecal Magnesium.—A comparison of the ratio of urinary magnesium to fecal magnesium demonstrates a marked difference in the case of advanced leprosy from that of the other subjects (Table XIX). In the normal individuals studied approximately one-third of the total magnesium excretion is by way of the urine. This relation holds fairly constant whether the individual is maintained on a low magnesium diet or whether additional magnesium has been administered. In general, with Case 1, on the other hand, almost as much or somewhat more magnesium is excreted by the urine than by the feces as long as the individual is on a magnesium-poor diet: When this element is added to the diet the ratio characteristic of the normal individual is obtained.

TABLE XIX.

Relation of Urinary and Fecal Magnesium.

Ratio: $\frac{\textit{Magnesium of Urine}}{\textit{Magnesium of Feces}}$.

Subject.	Period.			
	1	2	3	4
Subject 1; normal	0.6	0.5	0.5	0.6
" 2; "	0.5	0.4	0.4	0.4
Case 1; leper	1.2	1.2	0.7	0.6
" 2; "	0.4	0.4	0.4	0.4

A study of magnesium exchange in normal and leprous subjects demonstrates that in the advanced stage of leprosy there is a tendency for a retention of magnesium. This behavior is, however, not strikingly different from that of normal individuals maintained under similar dietary conditions.

Relation of Calcium to Magnesium.

Relation of Calcium and Magnesium in the Food.—In Table XX may be found the ratio of food calcium to food magnesium. Inspection of these data will demonstrate that in the first calcium-poor period, that is Period 1, the ratio is remarkably constant. In the second period, that in which calcium was added in the form of milk,

the ratio in the normal individuals changes markedly, but in the two instances is quite constant. With the leprosy subjects there is the same type of uniformity in the two individuals, but the ratio varies even further from that of Period 1 than in the normal subjects. With a return to a calcium-poor diet there is little variation in any subject from the figures for the first period. Added magnesium naturally lowers this ratio somewhat, but in no subject does it result in a perverted variation.

TABLE XX.

Relation of Calcium and Magnesium Intake.

Ratio: $\frac{\textit{Calcium of Food}}{\textit{Magnesium of Food}}$.

Subject.	Period.			
	1	2	3	4
Subject 1; normal	1.3	3.6	1.2	0.8
" 2; "	1.4	3.1	1.2	0.9
Case 1; leper	1.4	4.3	1.5	0.7
" 2; "	1.5	4.1	1.4	0.9

Relation of Calcium and Magnesium in the Urine.—In the first calcium-poor period, in spite of the fact that the calcium-magnesium ratio in the food was fairly constant, there is a considerable variation in the ratio of urinary elimination of these substances even in normal individuals (Table XXI).

TABLE XXI.

Relation of Calcium and Magnesium in the Urine.

Ratio: $\frac{\textit{Calcium of Urine}}{\textit{Magnesium of Urine}}$.

Subject.	Period.			
	1	2	3	4
Subject 1; normal	3.9	4.5	2.9	2.7
" 2; "	1.8	1.9	2.1	3.1
Case 1; leper	0.3	0.2	0.3	0.3
" 2; "	1.1	1.6	1.0	1.0

In the leprosy cases the ratio is distinctly perverted, especially in the advanced case. This perversion, however, may be explained by the retention of calcium by these subjects. When a calcium-rich diet is given the ratios vary more widely in the normal subjects than they do in the leprosy cases. Addition of magnesium does not alter the ratios in any determinable manner.

The most significant feature with respect to the ratio of calcium and magnesium in the urine is that a change from a calcium-poor diet to one rich in calcium, or the reverse, or a change from a magnesium-poor diet to a magnesium-rich diet does not induce any significant alteration in the unusual ratio of calcium to magnesium in the urine in the advanced case of leprosy, Case 1.

Relation of Calcium and Magnesium in the Feces.—As may be seen from inspection of Table XXII the relation between the calcium and

TABLE XXII.

Relation of Calcium and Magnesium in the Feces.

$$\text{Ratio: } \frac{\text{Calcium of Feces}}{\text{Magnesium of Feces}}.$$

Subject.	Period.			
	1	2	3	4
Subject 1; normal	1.5	3.7	1.2	1.4
" 2; "	1.8	3.0	2.1	1.3
Case 1; leper	2.5	4.6	3.1	1.5
" 2; "	1.5	3.0	1.3	1.0

magnesium of the feces in leprosy does not differ widely from that in normal individuals. The greatest variation is in Case 1 and is caused by the fact that relatively less magnesium is eliminated through the feces than in the other subjects.

A study of the relations existing between the calcium and magnesium of the food, urine, and feces demonstrates that even in normal subjects, although the relation of food calcium and magnesium may be quite constant, nevertheless a decided variation may exist in the relation of these elements in the urine and feces, especially in the former. This fact is undoubtedly explained by the extent of retention of one or the other of these elements.

In leprosy the perversion of ratios is much more noticeable, especially in the advanced case. Inasmuch as relatively large quantities of calcium and much smaller quantities of magnesium were retained in the body it is evident that the greater retention of these elements in leprosy is sufficient to explain the peculiar ratios observed, for instance, in Table XXI.

CONCLUSIONS.

In leprosy there is a definite retention of calcium.

The more advanced the stage of the disease the greater is the degree of retention.

This behavior on the part of leprous individuals may be taken as an indication that the organism is in need of calcium.

Magnesium may also be retained in leprosy, but the degree of retention is much less marked than in the case of calcium.

With the exception of the retention of calcium and magnesium the leprous organism responds to changes in the intake of calcium and magnesium in the same manner as normal individuals.

The results of this investigation suggest that in leprosy administration of calcium may be of benefit as an additional therapeutic measure in an endeavor to retard or arrest the progress of the bone changes characteristic of the disease.

STUDIES ON CALCIUM AND MAGNESIUM METABOLISM IN DISEASE.

II. Calcium and Magnesium Metabolism in Multiple Cartilaginous Exostosis.

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(Received for publication, February 16, 1920.)

Clinically, the bone changes occurring in multiple exostosis stand in sharp contrast to those involved in leprosy. In leprosy bone salts are lost from the body; in multiple exostosis bone salts are deposited. In a previous paper[1] we have shown that in leprosy calcium is retained to a marked extent. The same tendency to retain magnesium is present but not to the same degree. In other words, the diseased organism conserves its bone-building constituents.

In the present communication observations upon two selected cases of multiple exostosis are presented. As far as we are aware there are no records of an extensive study of the calcium and magnesium exchange in this proliferative type of bone disease.

Plan of Investigation.

The general plan of the experiment and the methods and dietary employed were identical with those outlined in the previous paper.

Description of Subjects.

Normal Individuals.

The individuals (Subjects 1 and 2) serving as controls in the investigation on leprosy have been taken as controls in the present observations also.

[1] Underhill, F. P., Honeij, J. A., and Bogert, L. J., *J. Exp. Med.*, 1920, xxxii, 41.

Exostosis Cases.

Case 1.—R W. H., male, single; age 31 years; farmer and later clerk.

Complaint.—Pain in knees; tires easily; limitation of motion.

Family History.—No evidence of heredity from grandparents' generation.

Past History.—Has had usual childhood diseases; in 1912 had nervous breakdown with gastric disturbances; had gonorrhea in 1916; no syphilis.

Present History.—At 6 years of age father noticed an enlargement on the inside of right ankle. 2 years later similar enlargement of left ankle. No pain or difficulty in walking. Later, from time to time until 21 years of age "lumps" were discovered all over body especially under left arm, axilla, and on ribs. Between ages of 10 and 15 years lumps appeared on lower extremities and on left hip after an injury. At same time fell on coccyx and later a lump developed there. At age of 23 noticed a lump on outer side, right thigh; this apparently was a result of a fall 2 years previous; at that time there were distinct ecchymoses over that area. Certain of lumps have disappeared. One on wrist disappeared 5 years after first noticed, others under arm, on left wrist, and on thumb. Never had fractures.

Physical Examination.—The patient is disproportionate in size, although muscular, is in general poorly developed, is short of stature, pigeon-breasted, and with rather prominent bony angles. Patient walks much like an automaton, with short jerky steps. Weight 114 pounds. The lungs, heart, and other organs are negative.

Examination of the bony skeleton shows numerous growths, of different sizes and apparently of different consistency. Some of these growths are easily grasped and felt and others are barely recognized. They occur more or less symmetrically. Those most easily felt and even seen occur on the femur, near knee joint, tibia, fibula, radius, and ulna. Near the shoulder on the left is a very prominent growth. There is a marked curvature of the forearms. The radiographs demonstrate these growths to be bony with terminal cartilage crowns. Some smooth and regular, others markedly irregular and rough. A large number occur near the epiphysis; some are undoubtedly responsible for the limitation of motion. The bones themselves are well formed with rather prominent and thickened epiphyseal portions.

The urine and blood examinations are negative.

Diagnosis.—Multiple cartilaginous exostosis; stationary.

Case 2.—C. G., male, single; age 16 years; tool maker.

Complaint.—Lumps on leg and crooked leg.

Family History.—No evidence of heredity in parents or children.

Past History.—Scarlet fever at 4 years of age.

Present History.—Patient has noticed lumps upon various bones, all his life, some of which have grown during last 6 years. During last few years has noticed that the right knee was becoming badly knocked. Never has had pain.

Physical Examination.—A fairly tall, rather slimly built young man; narrow shoulders and hips and with a decided right knock-knee; fairly well developed muscularly and fairly well nourished; weight 126 lbs. The lungs, heart, and other organs are negative.

Examination of the bony skeleton shows numerous growths or nodules most noticeably above and inside of right knee, also left knee. Other growths are also found on forearms near lower end. These growths are not so easily felt or so hard as in Case 1. The growths above the knees, however, are easily grasped and even seen.

The radiographs show many irregular growths, some long and spindle-shaped, others flat, rounded, and more like lumps. It is evident that these are bony growths with considerable cartilage. They are not so dense or definite in outline as those seen in Case 1. They occur mainly near and over the epiphysis. The bones themselves are apparently normal with abnormal epiphyses. Growths are seen on nearly all the bones.

The urine and blood examinations are negative.

Diagnosis.—Multiple cartilaginous exostosis; progressive.

Calcium and Magnesium Balances.

The data relative to the calcium and magnesium balances in Cases 1 and 2 may be found in Tables I and II. Those for the normal individuals, Subjects 1 and 2, are given in the previous paper.[2] In the first period on a diet low in calcium and magnesium Case 1, the more advanced case of exostosis, behaved with respect to the calcium balance in a manner very similar to the normal subjects, being in slight negative balance. On the other hand, when the magnesium balance exhibited by this subject is examined it will be found different from that for the normal subjects. With the latter a positive balance was obtained in the first period, but with the abnormal subject there is a very significant negative equilibrium. In the growing youth, Case 2, the negative calcium balance in the first period is marked, being three times greater than in Case 1. On the other hand, the negative magnesium balance in this period is much smaller than in Case 1.

Addition of calcium in the form of milk in the second period resulted in an approach to calcium equilibrium in all four cases. However, the added calcium did not materially alter the magnesium balance in either case of exostosis.

[2] Underhill, Honeij, and Bogert,[1] Tables II and III.

TABLE I.

Case 1; Exostosis. Calcium and Magnesium Balances.

Period.	Diet.	Date.	Calcium. Intake.	Calcium. Output. Urine.	Calcium. Output. Feces.	Calcium. Balance.	Magnesium. Intake.	Magnesium. Output. Urine.	Magnesium. Output. Feces.	Magnesium. Balance.
		1917	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1	Calcium-low and magnesium-low.	Jan. 23	573	180			366	110		
		" 24	537	418			362	125		
		" 25	563	175			354	114		
		" 26	500	321			336	136		
Total			2,173	1,094	1,938	−859	1,418	475	2,556	−1,623
				3,032				3,041		
Average per day			543	273	484	−214	354	118	639	−405
				758				760		
2	Calcium-high and magnesium-low.	Jan. 27	1,513	282			507	162		
		" 28	1,603	279			537	128		
		" 29	1,593	417			536	223		
Total			4,709	978	3,624	+107	1,580	513	2,507	−1,440
				4,602				3,020		
Average per day			1,569	326	1,208	+35	526	171	835	−480
				1,534				1,006		
3	Calcium-low and magnesium-low.	Jan. 30	483	188			345	92		
		" 31	450	138			322	151		
		Feb. 1	479	352			334	115		
Total			1,412	678	1,427	−693	1,001	358	2,401	−1,758
				2,105				2,759		
Average per day			470	226	475	−231	333	119	800	−586
				701				919		
4	Calcium-low and magnesium-high.	Feb. 2	517	386			550	177		
		" 4	522	129			573	118		
		" 4	522	217			553	180		
		" 5	516	279			554	207		
		" 6	459	161			541	141		
Total			2,536	1,172	1,418	−54	2,771	823	6,378	−4,430
				2,590				7,201		
Average per day			507	234	283	−11	554	164	1,275	−886
				518				1,440		

TABLE II.

Case 2; Exostosis. Calcium and Magnesium Balances.

Period.	Diet.	Date.	Calcium. Intake.	Calcium. Output. Urine.	Calcium. Output. Feces.	Calcium. Balance.	Magnesium. Intake.	Magnesium. Output. Urine.	Magnesium. Output. Feces.	Magnesium. Balance.
		1916	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1	Calcium-low and magnesium-low.	July 17	229	219			142	123		
		" 18	213	370			131	144		
		" 19	167	463			122	118		
		" 20	224	404			133	140		
Total			833	1,456	2,299	−2,922	528	525	379	−376
				3,755				904		
Average per day			208	364	574	−730	132	131	94	−94
				938				226		
2	Calcium-high and magnesium-low.	July 21	920	436			166	128		
		" 22	897	331			146	116		
		" 23	918	448			156	91		
Total			2,735	1,215	1,752	−232	468	335	315	−182
				2,967				650		
Average per day			911	405	584	−77	156	111	105	−60
				989				216		
3	Calcium-low and magnesium-low.	July 24	213	339			151	82		
		" 25	210	615			143	126		
		" 26	235	609			141	120		
Total			658	1,563	2,211	−3,116	435	328	246	−139
				3,774				574		
Average per day			219	521	737	−1,038	145	109	82	−46
				1,258				191		
4	Calcium-low and magnesium-high.	July 27	210	799			353	160		
		" 28	182	259			342	86		
		" 29	228	615			349	181		
Total			620	1,673	1,557	−2,610	1,044	427	483	+134
				3,230				910		
Average per day			206	557	519	−870	348	142	161	+44
				1,076				303		

The return to a diet low in calcium and magnesium in Period 3 caused all four subjects to assume a negative calcium balance, the two normal individuals and Case 1 behaving very much alike. Case 2, on the other hand, shows a very large negative calcium balance. When the magnesium balance is considered in this period it is found that the two normal subjects and Case 2 behave similarly; that is, show a slight negative magnesium balance. Case 1, on the other hand, exhibits a marked negative magnesium balance.

Placed in the fourth period upon a relatively high magnesium diet the two normal subjects remain in a significant negative calcium balance. With Case 1 equilibrium is practically attained whether considered from the standpoint of a 3 or 5 day period. Case 2, however, exhibits a marked negative balance. Addition of magnesium in the fourth period causes both normal subjects and Case 2 to change from a negative magnesium balance to a positive balance. In contrast to these results Case 1 exhibits an even greater negative balance than on a magnesium-free diet. This is true whether considered from the view-point of a 3 or 5 day period.

These facts demonstrate that in the exostosis cases there is a considerable variation in the manner in which the two subjects dispose of calcium and magnesium. Case 1 must be regarded clinically as a stabilized case of exostosis. This fact was demonstrated by comparative examinations 1 year apart. Case 2 is considered as in the progressive stage of the disease. From the metabolic view-point Case 1 behaves very much like a normal subject with respect to calcium exchange. A comparison of magnesium metabolism in this subject with that in the normal individuals shows that a significant negative balance is always present whether the subject is kept upon a low or high calcium or magnesium diet.

With the growing youth, Case 2, the calcium balance is always markedly negative whether the calcium intake is high or low. In other words, it would appear that this organism constantly attempted to rid itself of calcium. Negative magnesium balances are the rule also with this individual, except in the period of high magnesium intake. On the other hand, the negative magnesium is not at all comparable with that obtained with the stabilized case, No. 1.

These facts lead to the hypothesis that in exostosis in the progressive stage the organism shows an active effort to resist storage of calcium and magnesium. With the latter element, however, this tendency is not so marked. In the stabilized stage calcium exchange appears to approach the normal condition, but the loss of magnesium from the body is quite notable.

Further Analysis of the Data.

Calcium Metabolism.

The data obtained from the study of exostosis have been subjected to a further analysis in order to determine the correctness of the hypothesis stated above; namely, that in general in exostosis calcium and magnesium are eliminated from the body rather than stored.

Excretion of Added Calcium.—Calculation of the percentage of added calcium (Table III) eliminated from the organism in stabilized

TABLE III.

Excretion of Added Calcium.

Subject.	Excretion of added calcium.*
	per cent
Subject 1; normal	100+
" 2; "	92+
Case 1; exostosis	96+
" 2; "	?

* Period 2; calcium added as milk.

exostosis demonstrates a great similarity to that excreted by normal individuals under like experimental conditions. The percentage excretion of calcium in the growing patient, Case 2, is impossible to compute owing to the fact that the increased calcium intake appeared to check temporarily the excessive excretion of calcium characteristic of the subject upon a low calcium intake. In Periods 3 and 4 the massive excretion again prevails.

Relation of Calcium Output to Intake.—In Table IV may be found ratios of calcium excreted to calcium ingested for the four experimental

periods. These figures demonstrate that the stabilized case of exostosis behaved in a manner very similar to normal individuals. In Case 2, a developing boy without marked deposition of bone in growths, the metabolic rate is evidently different, since in the initial calcium-poor period the calcium output was more than four times the intake. It is, of course, possible that the low calcium intake acted as a stimulus to elimination of calcium from the body. Be this as it may, it is certain that when calcium was added to the diet in Period 2 the ratio is the same as that of normal individuals. In other words, this subject apparently had not lost the ability of disposing of calcium in a normal manner. When the addition of calcium

TABLE IV.

Relation of Calcium Output to Calcium Intake.

$$\textit{Ratio: } \frac{\textit{Calcium Output}}{\textit{Calcium Intake}}.$$

Subject.	Period.			
	1	2	3	4
Subject 1; normal	1.6	1.0	1.8	1.9
" 2; "	1.2	0.9	1.7	1.7
Case 1; exostosis	1.4	0.9	1.4	1.0
" 2; "	4.2	0.9	5.7	5.2

to the diet was discontinued the old ratio of output to intake again appeared, but to an even more exaggerated degree. It is probable that this is an expression of delay or lag in excretion, calcium either being retained somewhat temporarily or its introduction checking for a time the tendency to excessive elimination.

From the data it is evident that under the experimental conditions in active exostosis the organism is not tenacious of calcium. This fact may indicate that in its initial stages progress of the disorder may possibly be checked by proper restriction of the intake of calcium. It is, of course, impossible to draw such a deduction from a single instance, but the behavior of this individual under the experimental conditions imposed at least warrants the pursuit of such a lead.

Relation of Output to Intake for the Entire Period.—The relation of the total intake of calcium to its total output over the entire experimental period emphasizes the points discussed above (Table V).

Case 1, the stabilized case of exostosis, behaved very much like a normal individual. On the other hand, Case 2, the growing patient, showed a massive excretion.

TABLE V.

Relation of Calcium Output to Intake for the Entire Period.

Subject.	Calcium.		
	Output.	Intake.	Difference.
	mg.	*mg.*	*mg.*
Subject 1; normal	10,443	7,583	−2,860
" 2; "	12,626 (13 days).	9,752	−2,874
Case 1; exostosis	11,293 (13 ").	9,815	−1,478
" 2; "	13,726	4,846	−9,880

Relation of Calcium Intake and Output to Body Weight.—Inspection of Table VI will demonstrate at once the same general features of calcium metabolism already discussed. On the basis of weight Case 2 eliminated more calcium than any other individual, and yet the intake of calcium was not much more than one-half that of the others. Variations in body weight therefore will not account for the massive excretion observed with Case 2.

TABLE VI.

Relation of Calcium Intake and Output to Body Weight.

Subject.	Body weight.	Calcium.		Calcium per pound of body weight.	
		Intake.	Output.	Intake.	Output.
	lbs.	*mg.*	*mg.*	*mg.*	*mg.*
Subject 1; normal	105	7,583	10,443	72	99
" 2; " (13 days)	150	10,290	13,316	68	88
Case 1; exostosis	112	9,815	11,293	87	100
" 2; "	132	4,846	13,726	36	104

Absorption of Calcium.—Under the limitations outlined in the previous paper absorbed calcium is defined as the calcium eliminated through the urine; unabsorbed calcium that passing out with the feces. It may be seen from Table VII that the well advanced case of exostosis, Case 1, behaves like a normal person. On the other hand, with the growing boy, Case 2, the output of calcium through the urine on the calcium-poor diet (Period 1) was more than 1.5 times the intake, showing a distinct loss of calcium from the body tissues. Nevertheless, on a calcium-rich diet (Period 2) this individual exhibits a behavior similar to that of a normal person, but when he was again placed upon a calcium-poor diet (Periods 3 and 4) the calcium

TABLE VII.

Relation of Urinary Calcium Excretion to Calcium Intake.

Ratio: $\frac{\text{Calcium of Urine}}{\text{Calcium of Food}}$.

Subject.	Period.			
	1	2	3	4
Subject 1; normal	1.0	0.4	1.0	1.0
" 2; "	0.4	0.2	0.5	0.8
Case 1; exostosis	0.5	0.2	0.4	0.4
" 2; "	1.7	0.4	2.3	2.7

excreted by the urine was more than twice that ingested. This excessive excretion occurred in spite of the fact that there was in this individual an enormous quantity of cartilage being laid down and that there would be accordingly a great demand for bone salts.

Relation of Fecal Calcium to Calcium Intake.—A consideration of the relation of calcium in the feces to calcium ingested shows that in the exostosis patients the same type of behavior as discussed above holds true (Table VIII). The only deviation from the normal excretion of calcium by way of the feces to be observed is that in Case 2. On a calcium-low diet (Period 1) more than 2.5 times the calcium of the food is eliminated by way of the intestine. This does not indicate any inability to absorb calcium, for on a calcium-rich diet (Period 2) the calcium excreted through the feces assumes a

relation to the calcium of the food of normal proportions. Resumption of a calcium-poor diet causes the reappearance of the same unusual degree of calcium excretion observed in the first period.

TABLE VIII.

Relation of Fecal Calcium to Calcium Intake.

Ratio: $\frac{\text{Calcium of Feces}}{\text{Calcium of Food}}$.

Subject.	Period.			
	1	2	3	4
Subject 1; normal	0.6	0.6	0.8	0.8
" 2; "	0.8	0.7	1.1	0.8
Case 1; exostosis	0.9	0.7	1.1	0.5
" 2; "	2.7	0.6	3.3	2.5

Relation of Urinary Calcium to Fecal Calcium.—If a comparison is made between the output of calcium in the urine and feces it is apparent (Table IX) that in the cases of exostosis this relation does not deviate very markedly from that of normal individuals.

TABLE IX.

Relation of Urinary Calcium to Fecal Calcium.

Ratio: $\frac{\text{Calcium of Urine}}{\text{Calcium of Feces}}$.

Subject.	Period.			
	1	2	3	4
Subject 1; normal	1.5	0.6	1.1	1.1
" 2; "	0.5	0.2	0.4	1.0
Case 1; exostosis	0.5	0.2	0.4	0.8
" 2; "	0.6	0.6	0.7	1.0

In a stabilized case of multiple exostosis the calcium exchange differs little from that of a normal individual whether the abnormal subject is maintained upon a calcium-poor diet or upon one rich in this element. In a growing individual with multiple exostosis the calcium exchange is markedly different from that of a normal indi-

vidual. On a calcium-poor diet calcium is lost from the body in relatively large quantity, being eliminated by both the urine and feces in a perfectly normal ratio. On a calcium-rich diet calcium is retained to an extent not widely deviating from that in normal persons. A resumption of a calcium-poor diet again induces excessive calcium elimination. These facts suggest a possible dietary procedure to check the further development of the abnormal condition through deposit of calcium in early cartilaginous growths.

Magnesium Metabolism.

Excretion of Added Magnesium.—The figures in Table X show how promptly added magnesium is excreted in subjects with multiple exostosis. Under like experimental conditions normal individuals

TABLE X.

Excretion of Added Magnesium.

Subject.	Excretion of added magnesium.*
	per cent
Subject 1; normal	36
" 2; "	45
Case 1; exostosis	100
" 2; "	80

* Period 4; magnesium added as citrate.

retain more than one-half the added magnesium, whereas in the abnormal cases the excretion is much larger. With the stabilized case of exostosis, Case 1, 100 per cent of the added magnesium was quickly eliminated. The less advanced case, Case 2, excreted about 80 per cent of the introduced magnesium. This may perhaps be explained by the view that the stabilized case had no unusual demand for bone-building salts, whereas in the young, growing, active subject, the calcification of growths had not progressed to a marked degree.

Relation of Magnesium Output to Intake.—Considerable interest attaches to the relation which exists between magnesium output and intake, especially in Case 1, the well stabilized case of exostosis

(Table XI). On a diet low in magnesium the output is twice as great as the intake and indeed at times, as in Period 3, may be almost three times as great. Added magnesium does not change this relation. This type of case, therefore, either is unable to retain magnesium or else the body attempts to rid itself of it. The latter view is undoubtedly the more probable explanation, since the magnesium excretion is constantly three times greater than the intake.

With the less advanced case, Case 2, the same type of behavior relative to magnesium may be observed, although the degree of excretion is not so marked. This case in general more nearly approaches normal behavior, and when magnesium is added there is

TABLE XI.

Relation of Magnesium Output to Intake.

$$\textit{Ratio: } \frac{\textit{Magnesium Output}}{\textit{Magnesium Intake}}.$$

Subject.	Period.			
	1	2	3	4
Subject 1; normal	0.9	0.9	1.2	0.7
" 2; "	0.9	1.1	1.0	0.8
Case 1; exostosis	2.1	1.9	2.7	2.6
" 2; "	1.6	1.4	1.3	0.9

distinct retention. This fact leads to the query whether the results obtained with this subject are explicable on the hypothesis that the calcium intake was too small for the individual to be able to reach equilibrium. Such a view is pertinent, since clinically the patient was in apparent need of both magnesium and calcium for the maintenance of general growth. Moreover, the new growths were abundant and progressive. The fact that added calcium brought this subject into balance favors the hypothesis stated above. On the other hand, distinctly against this view-point is the fact that as the experimental period advanced, equilibrium was more nearly approached, although the magnesium intake became progressively lower. Inasmuch as inadequate magnesium intake cannot be ascribed as the cause of the peculiar behavior of Case 1, the stabilized case, and, moreover, since the general features of both cases are very simi-

lar, it seems logical to conclude that the deviation from normal observed in the less advanced case, Case 2, is to be attributed to the stage of the disease rather than to an inadequate magnesium supply.

Relation of Output to Intake for the Entire Period.—The figures in Table XII, showing the relation of magnesium output to intake for the total experimental period, merely corroborate and emphasize the points discussed above.

TABLE XII.

Relation of Magnesium Output to Intake for the Entire Period.

Subject.	Magnesium.		
	Output.	Intake.	Difference.
	mg.	*mg.*	*mg.*
Subject 1; normal	4,133	4,468	+335
" 2; " (13 days)	5,806	5,894	+88
Case 1; exostosis (13 ")	13,140	5,661	−7,479
" 2; "	3,038	2,475	−563

Relation of Magnesium Intake and Output to Body Weight.—An inspection of Table XIII will show at once that the variability of body weight will not explain the differences observed previously with respect to magnesium exchange in multiple exostosis. Moreover, when the intake and output of magnesium are considered in relation to body weight it is at once apparent that insufficient intake cannot explain the variations. Thus, Case 1 ingested a relatively greater quantity of magnesium than any of the other subjects and yet the

TABLE XIII.

Relation of Magnesium Intake and Output to Body Weight.

Subject.	Body weight.	Magnesium.		Magnesium per pound of body weight.	
		Intake.	Output.	Intake.	Output.
	lbs.	*mg.*	*mg.*	*mg.*	*mg.*
Subject 1; normal	105	4,468	4,133	42	39
" 2; " (13 days)	150	5,894	5,806	39	38
Case 1; exostosis (13 ")	112	5,661	13,140	50	117
" 2; "	132	2,475	3,038	18	23

excretion was enormous. It is perhaps possible that with Case 2 a small intake of magnesium may play a part in the results noted. On the other hand, this seems hardly probable, since in general the behavior of this individual with respect to magnesium exchange resembled that of Case 1.

Absorption of Magnesium.—A comparison of the relation existing between urinary magnesium and food magnesium in normal individuals and in cases of multiple exostosis shows a striking similarity in all instances except one; namely, Case 2. Here the urinary magnesium excretion on diets low in magnesium varies from 0.7 to 0.9 of the intake instead of 0.3 to 0.4 as with normal subjects (Table XIV). Addition of magnesium to the diet causes this relation to

TABLE XIV.

Relation of Urinary Magnesium Excretion to Magnesium Intake.

Ratio: $\frac{\text{Magnesium of Urine}}{\text{Magnesium of Food}}$.

Subject.	Period.			
	1	2	3	4
Subject 1; normal	0.3	0.3	0.4	0.2
" 2; "	0.3	0.3	0.3	0.2
Case 1; exostosis	0.3	0.3	0.3	0.3
" 2; "	0.9	0.7	0.7	0.4

approach the higher limits exhibited by the normal subjects. From these facts it is evident that in the abnormal cases under observation magnesium absorption was not inferior to that in normal individuals.

Relation of Fecal Magnesium to Magnesium Intake.—A consideration of the relation of excretion of magnesium by way of the feces to ingestion of this element shows that only in Case 1 is the ratio of fecal magnesium to food magnesium outside the normal limits (Table XV). With Case 1 there is a massive excretion of magnesium by way of the intestine. Inasmuch as this excretion is far greater than the intake, and since the absorption of magnesium is normal, it follows that the excess magnesium eliminated comes from the tissues, the intestine being the principal path of elimination.

TABLE XV.

Relation of Fecal Magnesium to Magnesium Intake.

$$Ratio: \frac{Magnesium\ of\ Feces}{Magnesium\ of\ Food}.$$

Subject.	Period.			
	1	2	3	4
Subject 1; normal	0.5	0.6	0.8	0.4
" 2; "	0.6	0.8	0.7	0.5
Case 1; exostosis	1.8	1.5	2.9	2.3
" 2; "	0.7	0.7	0.6	0.5

Relation of Urinary Magnesium to Fecal Magnesium.—From the data in Table XVI it is apparent that the ratio of urinary magnesium to magnesium of the feces is different from that exhibited by normal persons. In the normal subjects approximately twice as much magnesium is excreted by way of the intestine as through the urine. This relation holds fairly constant whether the individual is main-

TABLE XVI.

Relation of Urinary Magnesium to Fecal Magnesium.

$$Ratio: \frac{Magnesium\ of\ Urine}{Magnesium\ of\ Feces}.$$

Subject.	Period.			
	1	2	3	4
Subject 1; normal	0.6	0.5	0.5	0.6
" 2; "	0.5	0.4	0.4	0.4
Case 1; exostosis	0.2	0.2	0.1	0.1
" 2; "	1.4	1.1	1.3	0.8

tained upon a low magnesium diet or whether magnesium has been added to the diet. In the stabilized case of exostosis, Case 1, the fecal excretion of magnesium is five to seven times that of the urinary elimination. With the less advanced case, Case 2, conditions are reversed. The urinary excretion of magnesium exceeds somewhat the elimination by way of the feces with a single exception. This occurs in Period 4, during which magnesium citrate was added to the

food. Under these circumstances magnesium elimination through the feces was slightly higher than excretion through the urine. In this period, therefore, Case 2 approaches quite closely the normal type in excretion of magnesium. From the observations it is evident that in multiple exostosis there is a derangement of the excretory ratio for magnesium, the deviation from normal being in either direction. Thus, in stabilized multiple exostosis magnesium is lost from the body principally by the intestine. When the progress of the disease has not become stabilized it is indicated that the greater portion of magnesium excreted goes by way of the kidneys.

In a stabilized case of multiple exostosis magnesium added to a diet low in this element is promptly excreted. Magnesium elimination in a less advanced case follows a similar course but to a less marked degree. These facts may be interpreted to mean that in the stabilized case there was no unusual demand for bone-building salts, whereas in the more active stage of the disease the degree of calcification in growths had not progressed to a marked extent, hence more magnesium was retained. In the stabilized case of exostosis, magnesium output was two to three times greater than the intake whether the subject was maintained upon a diet poor or rich in magnesium. With the less advanced case the ratio of excretion to intake follows closely that of the stabilized case, but the degree of excretion is not so marked. When magnesium is added there is distinct evidence of retention. The variations in magnesium exchange cannot be attributed to variable body weight. The absorption of magnesium in individuals with multiple exostosis is not inferior to that in normal subjects. Inasmuch as neither inadequate intake nor faulty absorption will account for the excessive elimination of magnesium in multiple exostosis it is obvious that it has its origin in the body tissues. From the observations detailed above it is apparent that in multiple exostosis there is a derangement of the excretory ratio for magnesium, the deviation from normal being in either direction. In the stabilized condition magnesium is lost from the body principally by way of the intestine, whereas in the more active stage of the disease the larger portion of magnesium excreted is by the kidneys.

Relation of Calcium to Magnesium.

Relation of Calcium and Magnesium in the Food.—In a consideration of the metabolism of either calcium or magnesium it is of importance to determine the relation existing between these two elements in the food and in the excretion, since there is some evidence that the intake of one may influence the elimination of the other. It is at once apparent from Table XVII that in the first period all four subjects exhibited a strikingly constant ratio of calcium to magnesium in the food. When calcium was added to the diet, only in Case 2 was there a deviation. Apparently in this instance the calcium intake was greatly increased without a corresponding augmentation of

TABLE XVII.

Relation of Calcium and Magnesium Intake.

$$\text{Ratio: } \frac{\text{Calcium of Food}}{\text{Magnesium of Food}}.$$

Subject.	Period.			
	1	2	3	4
Subject 1; normal	1.3	3.6	1.2	0.8
" 2; "	1.4	3.1	1.2	0.9
Case 1; exostosis	1.3	3.0	1.4	0.9
" 2; "	1.5	5.8	1.5	0.6

magnesium. Throughout the remainder of the investigation the ratio is not notably peculiar in any instance. It is therefore evident that, in general, all subjects received approximately the same proportions of calcium and magnesium.

Relation of Calcium and Magnesium in the Urine.—From an inspection of Table XVIII it may be seen that although the calcium-magnesium ratio of the food of all subjects was fairly constant a marked variation occurs even in the normal subjects in the relation of calcium and magnesium in the urine. With the stabilized case of exostosis the variations observed are within normal limits. Case 2, however, shows constantly a decided tendency to go beyond the normal limits. In general, the ratio of calcium to magnesium is higher than with the other subjects.

TABLE XVIII.

Relation of Calcium and Magnesium in the Urine.

$$\text{Ratio: } \frac{\text{Calcium of Urine}}{\text{Magnesium of Urine}}.$$

Subject.	Period.			
	1	2	3	4
Subject 1; normal	3.9	4.5	2.9	2.7
" 2; "	1.8	1.9	2.1	3.1
Case 1; exostosis	2.2	1.9	1.9	1.4
" 2; "	2.7	3.6	4.7	3.9

Relation of Calcium and Magnesium in the Feces.—The stabilized case of exostosis, Case 1, shows that magnesium is eliminated by the intestine in excessive amounts when considered relative to the calcium excretion (Table XIX, Period 1). Addition of calcium to the diet changes the ratio so that it is more nearly comparable with the normal ratio, although still far from it. This change is effected by the additional calcium eliminated and without any marked influence

TABLE XIX.

Relation of Calcium and Magnesium in the Feces.

$$\text{Ratio: } \frac{\text{Calcium of Feces}}{\text{Magnesium of Feces}}.$$

Subject.	Period.			
	1	2	3	4
Subject 1; normal	1.5	3.7	1.2	1.4
" 2; "	1.8	3.0	2.1	1.3
Case 1; exostosis	0.8	1.4	0.6	0.2
" 2; "	6.0	5.5	9.0	3.2

upon the magnesium excretion. Resumption to a low calcium diet again causes a return to the previous ratio. Addition of magnesium (Period 4) does not influence the output of calcium in the feces, and hence the ratio of calcium to magnesium is significantly decreased.

When the ratio of calcium to magnesium in the feces is considered with Case 2 the results are just the opposite of those observed with

Case 1. In other words, calcium is excreted by the intestine in excessive amounts (Period 1), the ratio not being materially altered even on a calcium-rich diet (Period 2). On returning to a calcium-poor diet (Period 3) again the ratio is greatly increased, apparently an indication of a lag in the rate of excretion. Addition of magnesium to the diet (Period 4) considerably lowers the ratio. This is caused by two factors, a somewhat lower calcium excretion and an increased magnesium output by the intestine.

The very diverse behavior exhibited by these two cases of exostosis leads one to query whether this difference is indicative of two stages in the abnormal metabolic process. That is, in the early stage calcium is more readily eliminated from the body than magnesium. In the stabilized condition, on the other hand, the reverse is true. Calcium is retained in a more or less normal manner but the relative excretion of magnesium is excessive. It is possible that in these two stages calcium and magnesium play a distinctive part in the calcification of the new growths, one element being more in demand at one period than at another.

The observations recorded above indicate that in general the relation of calcium and magnesium in the dietary of the experimental subjects was approximately the same for all subjects. Variations in excretion, therefore, cannot be explained on the hypothesis that varying proportions of one or the other element are responsible for the alterations in elimination previously noted. In general, in the early stage of exostosis calcium is excreted much more freely than magnesium, whereas in the stabilized stage of the disease magnesium is eliminated in excessive amount. In the stabilized case of exostosis the excessive excretion of magnesium is manifested through the intestinal path. On the other hand, with the less advanced case, Case 2, excessive elimination of magnesium is seen in both the urine and the feces. Neither in normal individuals nor in the cases of exostosis studied does addition of calcium or magnesium materially influence the excretion of the other element.

CONCLUSIONS.

In the stabilized stage of exostosis calcium exchange differs little from that of a normal individual whether the abnormal subject is maintained upon a calcium-poor diet or upon one rich in this element.

In the progressive stage of the disease calcium metabolism is markedly different from the normal in that calcium is lost from the body in large amounts when the subject is maintained upon a calcium-poor diet. This excessive elimination of calcium is by way of both the urine and feces in a normal ratio. When placed upon a calcium-rich diet calcium is retained to an extent not widely deviating from that obtained in normal subjects. A resumption to a calcium-poor diet again induces excessive calcium elimination.

In the stabilized stage of exostosis magnesium excretion is two or three times greater than the intake whether the subject is maintained upon a diet poor or rich in magnesium.

In the progressive stage of the disease the general type of magnesium excretion resembles that of the stabilized stage but the degree of elimination is not so marked.

Magnesium added to the diet in the stabilized stage is promptly excreted. The same test applied to the progressive stage gives evidence of retention of some magnesium. The degree of retention, however, is much less than that shown by normal individuals.

Absorption of both calcium and magnesium in exostosis is not inferior to that of normal subjects.

The facts enumerated suggest that in the early stages of exostosis, that is during the proliferative cartilage changes, the progress of the disease may perhaps be checked by proper dietary procedure—restriction of calcium and magnesium intake.

A FURTHER EXPERIMENTAL STUDY ON EXCITATION OF INFECTIONS OF THE THROAT.

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(Received for publication, April 1, 1920.)

In experiments previously published it has been shown that chilling of the body surface causes vasoconstriction and ischemia—not, as theretofore assumed, congestion—in the mucous membranes of the palate, pharynx, and palatine tonsils.[1] Upon rewarming the subject this ischemia was shown to disappear only partially from the palate and pharynx, but, in a single experiment upon the palatine tonsil, promptly gave way to an actual hyperemia. Because of the peculiar importance of the tonsils as foci of infection, it was considered necessary to devote further study to their reactions. The present experiments amply confirm the occurrence in the tonsils of the reaction of ischemia to chilling of the body surface; a composite of the four recovery curves obtained bears out also the first observation that the tonsils, upon the subject's being rewarmed after chilling, show a considerably greater tendency than do the contiguous mucous membranes of the palate and pharynx to regain a full blood supply.

Concurrent bacteriological observations on the pharynx and tonsils of several of the subjects showed after the experiments in several instances apparently an increase in the relative number of some of the pathogenic bacteria, synchronous with the appearance of a clinical sore throat.

Urine analysis indicated secretion during an experiment of a more dilute and less acid urine. In no instance was albuminuria or glycosuria produced.

[1] Mudd, S., and Grant, S. B., *J. Med. Research*, 1919, xl, 53.

Method.

The thermopile method described in the earlier paper[2] was again used, but with several notable improvements in detail. The subject entered the experimental room, kept between 16.9° and 20.9°C., warmly wrapped in loose garments. Thoracic and abdominal pneumographs were adjusted to a moving drum writing in the subject's view. The terminals of a thermopile, borne on a carrier of galvanized iron wire, were strapped by adhesive tape to the subject's forehead. The terminals of a second thermopile were fixed by means of another galvanized iron applicator and a specially devised holder, to be described below, in apposition with the mucous membrane of one palatine tonsil. Thereafter the subject maintained his position as nearly as possible constant throughout the experiment. The proximal ends of the thermopiles were kept packed in cotton in a test-tube with the bulb of a sensitive thermometer, and were connected through a rocking key with a d'Arsonval galvanometer.

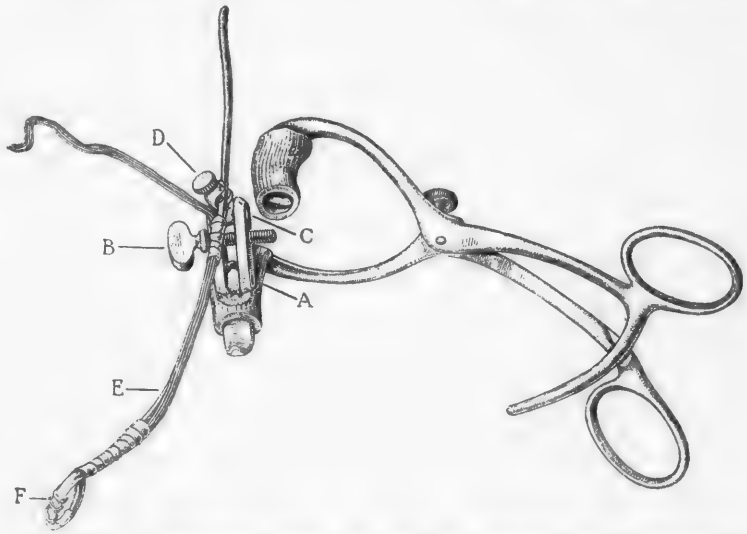
The subject's nostrils were plugged with cotton; breathing was through the open mouth. At least a part of the difficulty introduced by the excessive secretion of saliva under these circumstances was eliminated in the present experiments by keeping in the subject's mouth a glass saliva ejector of the type used by dentists, connected through a rubber tube to a suction pump on a water faucet.

When thermopiles, pneumographs, and saliva tube had once been properly adjusted, readings of galvanometer and thermometer were made at about 60 second intervals for a few minutes. Respiration was then deepened by an amount sufficient to increase the excursion of the pneumograph levers on the average for the experiments illustrated in the charts by about 73 per cent, the respiratory rate being kept constant throughout the experiment with the aid of a metronome. With the intake of cool room air thus increased, mucous membrane temperature usually fell slightly. When the readings showed themselves approximately constant, the subject's wraps were all removed and an electric fan was turned at short range on the lumbar region of the back; chilling continued in the experiments illustrated in the charts from 7 to 19 minutes, with an average duration

[2] Mudd and Grant,[1] pp. 54–60.

of 13.8 minutes. The subject was finally rewrapped and readings were continued until the end of the experiment.

From the thermometer and galvanometer readings thus made, skin and mucous membrane surface temperatures are readily computed and their changes accurately followed. It is to be borne in mind that these temperature changes under the conditions of the experiments have been shown to be satisfactory indices of local vasomotor changes.[3] Therefore, although the curves in the text-figures actually record



TEXT-FIG. 1. Applicator holder with tonsillar applicator and thermopile in position. *A* and *C*, ball and socket joints made by metal spheroids and blades of head mirror; *B*, set screw for tightening joints at *A* and *C*; *D*, set screw for fastening applicator in holder; *E*, galvanized iron wire applicator; *F*, applying surface bearing thermopile terminals.

variations in superficial temperature of the skin and exposed mucous membranes, what they indicate is alteration in vasomotor tone—a fall in temperature means local vasoconstriction and ischemia, a rise local vasodilation.

The most important improvement in method in the present study has been the devising of a special instrument by which accurate and

[3] Mudd and Grant,[1] pp. 58–60.

stable adjustment of the thermopile terminals to the tonsillar surface, or other site in mouth or throat, can be effected. Into one arm of a Doyen mouth gag (Text-fig. 1) a small brass spheroid was screwed. A second brass spheroid bearing a groove closed by a set screw (*D*) was made. The two spheroids were connected by means of the blades of an ordinary head mirror (*C*). The blades could be clamped stably upon the spheroids by means of a second set screw (*B*). The applicator (*E*) so shaped as to have its tip fit against the tonsil, was held in place in the groove by the set screw *D*. By varying the shape and position of the applicator and arranging properly the joints at *A* and *C* any desired application could be made. The subject's teeth were protected from the metal gag by rubber, as shown in the drawing.

As is evident, the iron wire applicators used with this new holder are somewhat simpler than the original types; they are also of slightly lighter wire; No. 13 has been found most satisfactory.

The subjects used, as in the earlier experiments, were all upper class medical students, except J. D. R. and B. J. F. in the first and W. G. E. in the present series, who were laboratory employees several years younger.

For calibration the unknown temperature terminals of the thermopiles were bound with elastic about the bulb of a sensitive thermometer, which was immersed in a suspended test-tube of distilled water or salt solution. In the earlier experiments heat in small amounts was applied at short intervals directly to the test-tube. The contained liquid was stirred, and, when equilibrium had been reached, the galvanometer deflection and temperatures of the two ends of the thermopiles were read. From these data the calibration curves for the thermopiles were constructed. Since these were apparently, within the temperature range of the experiments, virtually straight lines, the results were averaged for each thermopile and the calibration constants thus obtained; *i.e.*, the straight line $x = ky$, when y is the galvanometer deflection in millimeters, x the temperature difference in °C. between the two ends of the thermopiles, and k the calibration constant, in each case with a value of approximately 0.1.

In the experiments of the present series the test-tube of water containing the thermopile terminals and the thermometer was immersed in addition in a suspended beaker of water containing a stirrer, and heat was applied to the beaker at intervals. The water in the beaker and that in the test-tube were then stirred until a constant temperature had been reached, and the thermometer and galvanometer readings then taken. With the more accurate results thus obtained,

the calibration curves were found to deviate appreciably, though very slightly, from straight lines. Temperatures were therefore taken from the curves directly.

The deviations were such that with the larger deflections the temperatures calculated from the calibration constant were slightly higher than those actually shown by the curve; with the smaller deflections calculated temperatures were slightly too low. For instance, to take extreme values, with a galvanometer deflection of 206 mm., the thermopile constant temperature end being 17.75°C., the mucous membrane temperature calculated from the calibration "constant" (in this case 0.09904) is 38.15°C., that shown by the curve, 37.43°C. With a galvanometer deflection of 56 mm. and constant end at 18.5°, the calculated mucous membrane temperature is 24.05°, that shown on the curve is 24.47°C. The high mucous membrane temperature values mentioned previously[4] are thus at least in large part accounted for. To be corrected the curves of the first paper should be very slightly foreshortened along the vertical axis; however, the error introduced was obviously quite negligible from the point of view for which the curves were designed; *i.e.*, to show temperature variations through a small range.

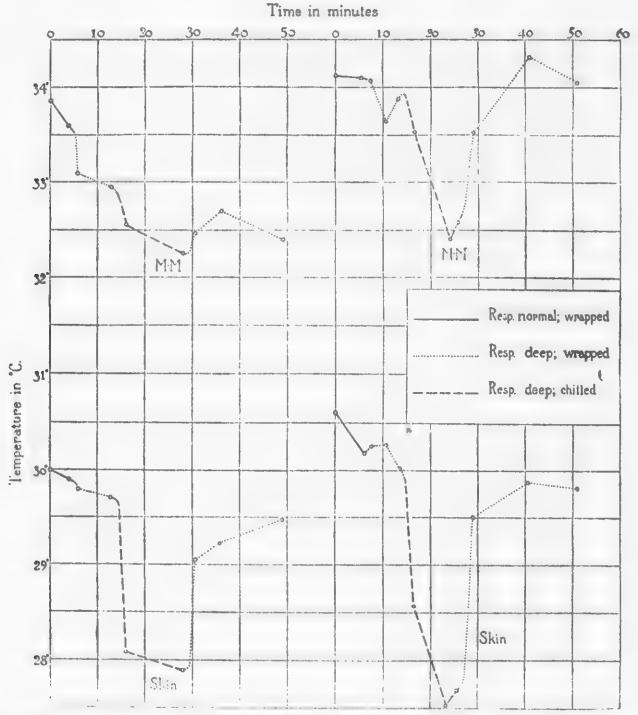
Vasomotor Reactions of Palatine Tonsils.

Composite Graphs.—The characteristic reactions of skin and mucous membrane to chilling of the body surface are shown in Text-fig. 2. The curves on the right are a composite of four experiments upon three different subjects; for the mucous membrane curve the thermopile tips were applied to the palatine tonsil surface; the synchronous lower curve is from the skin of the forehead. The curves on the left are a composite of three experiments upon the forehead, and, in two instances, mucous membrane of soft palate, in one instance, mucous membrane of oropharynx; two subjects were used. In each case the first and last point of the experiment, the point immediately before and after a change of conditions, and the point of maximum mucous membrane response to rewrapping were taken, and from their average values the curves were drawn. In the right hand curve the two points of maximum mucous membrane response to deepened respiration and to chilling are also included.

The palatine-oropharyngeal curve underwent the usual slight depression with deepened respiration. Upon chilling, the mucous membrane temperature fell 0.69°C. in 14 minutes; the corresponding point on the skin curve shows a depression of 1.80°. Upon rewrapping,

[4] Mudd and Grant,[1] p. 55.

the mucous membrane temperature rose in 7.3 minutes 0.40°, thereafter sinking slightly. The skin curve showed a maximum rise of 1.60° in 14.8 minutes. The maximum recovery exhibited within a 20 minute period after rewrapping was, then, for the palatine-oropharyngeal curve only 58 per cent of the fall in response to chilling; for the skin curve recovery was 88.8 per cent of the fall.



TEXT-FIG. 2. Reactions to chilling and to rewarming. On left, temperatures of skin and mucous membranes (*M.M*) of soft palate and oropharynx (composite graphs of Experiments 10, 12, and 13); on right, temperatures of skin and palatine tonsil (composite graphs of Experiments 1, 3, and 5 of the present series and Experiment 20 of the earlier series[5]).

[5] Mudd and Grant,[1] p. 85.

The palatine mucous membrane and skin curves responded to chilling by the usual depression, amounting to 1.47° for tonsil and 2.49° for skin. On rewarming, the mucous membrane reached a maximum rise of 1.72° in 14.1 minutes, the skin a rise of 2.33° in 14.5 minutes. Recovery was thus 117 per cent for tonsillar mucous membrane, 93.6 per cent for skin.

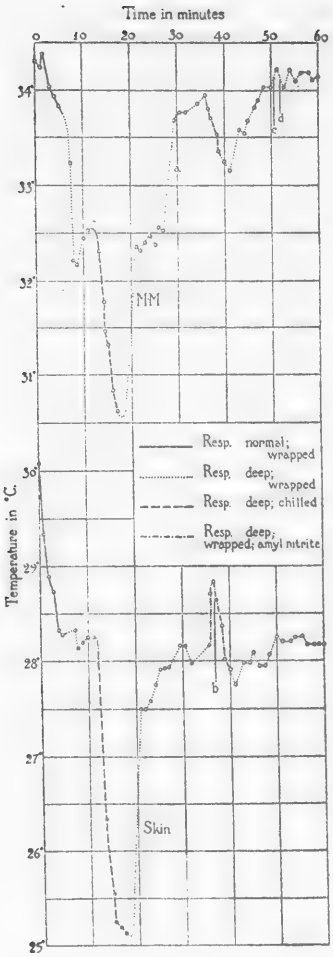
It is to be noted that, because the subject could not change position during the experiment and because of all the apparatus attached to him, he was practically never quite so warmly wrapped after chilling as he had been before. If conditions had been made identical before and after, doubtless all the recovery curves would have been somewhat higher.

Evidently the tonsils tend strongly to react to rewarming after chilling by becoming hyperemic, whereas the pharynx and palate, as was also shown in the earlier experiments, tend to remain somewhat ischemic for a considerable period after chilling. The skin occupies an intermediate position and returns approximately to its original condition.

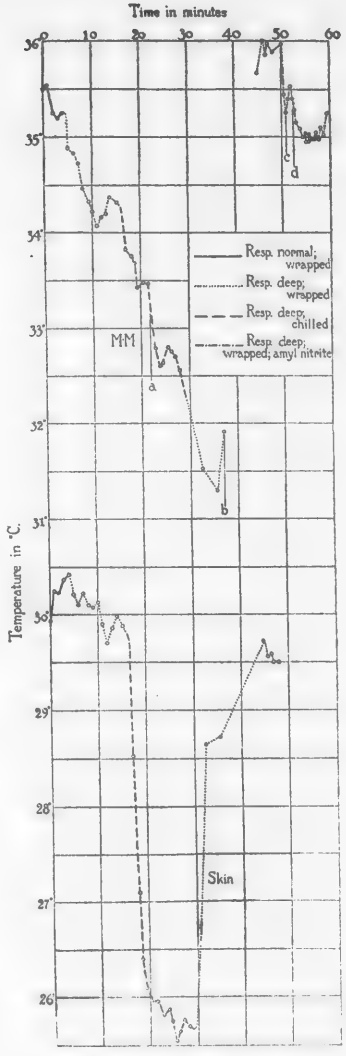
Experiment 1.—Subject 1, W. G. E. June 11, 1919, 11 a.m. to 12 m. Mucous membrane thermopile on left tonsil. Skin thermopile on forehead. Respirations 14 per minute. Mouth open; nostrils plugged. Thoracic and abdominal pneumographs. Room temperature 17.30–18.05°C.

Text-fig. 3 shows strikingly the characteristic tonsillar reactions. When the mucous membrane temperature had adjusted itself to deep respiration, chilling was begun at 0:12.[6] By 0:17 the mucous membrane temperature had fallen 1.91°, the skin temperature 3.11°. The subject was rewrapped at 0:17.5. 17 minutes later the tonsillar temperature had risen 3.24°, the skin temperature 3.04°. At 0:35 inhalation of an ampule of amyl nitrite was begun. The short transient rise in skin temperature, corresponding to the visible flush, at once developed; the peak, 0.66° higher, was reached in about 1.5 minutes, the maximum secondary fall to 0.42° below the original level in 6 minutes. The mucous membrane temperature, on the

[6] 0:12, 0:17, etc., indicate the time after the beginning of the experiment; *i.e.*, 0:12 signifies 12 minutes after the beginning of the experiment, 0:17, 17 minutes, etc.



TEXT-FIG. 3.



TEXT-FIG. 4.

TEXT-FIG. 3. Reactions to chilling, rewarming, and amyl nitrite. Temperatures of skin and mucous membrane of faucial tonsil, Experiment 1. *a*, applicator noted to be properly adjusted; *b*, flush of face fading; *c*, breathing shallow; *d*, breathing deeper.

TEXT-FIG. 4. Reactions to chilling, rewarming, and amyl nitrite. Temperatures of skin and mucous membrane of faucial tonsil, Experiment 2. *a*, fan faster; *b*, applicator loose; readjusted; *c*, face flushed; *d*, face fairly white.

other hand, which was already at the high level of 33.86°, fell with amyl nitrite to 33.17°, reached after 6 minutes, and then rose to a level slightly above 34° about 15 minutes after inhalation.

The tonsil here responded to amyl nitrite in the same way as did an acutely inflamed soft palate.[7] The mucous membrane vessels were already dilated; the effect of the relaxing of the mucous membrane blood vessel walls by amyl nitrite must have been overshadowed by the general lowering of blood pressure, the rate of circulation through the mucous membrane vessels was decreased, and the temperature fell. It is possible that increased depth of respiration with amyl nitrite also contributed to the observed fall.

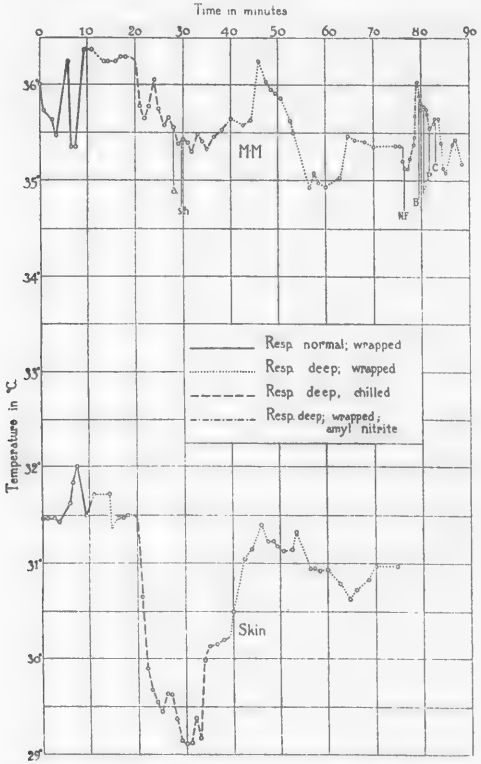
Experiment 2.—Subject 2, L. J. O. June 7, 1919, 10.30 to 11.30 a.m. Mucous membrane thermopile on left tonsil. Skin thermopile on forehead. Respirations 14 per minute. Mouth breathing. Thoracic and abdominal pneumographs. Room temperature 17.12–17.87°C.

Text-fig. 4 shows the usual depression of skin and mucous membrane temperatures; the latter in this case continued to fall for about 6.5 minutes after cessation of chilling, possibly because of faulty adjustment of the applicator, which at 0:38 was noted to be loose, and was readjusted. The next reading showed a mucous membrane temperature 1.37° above the last reading before chilling. This curve thus probably records a reaction of hyperemia to rewrapping, but it was excluded from the composite graph because of the readjustment of the thermopile. On inhalation of amyl nitrite at 0:49, the response was again a drop in tonsillar temperature—0.94° in 6 minutes; the increase in respiratory amplitude was at most 30 per cent. The increase in respiratory amplitude at 0:4.5 amounted to 84.6 per cent and was followed within 6.5 minutes by a depression of only 1.16°. The temperature fall after amyl nitrite was, then, evidently due in part both to respiratory increase and to general blood pressure fall.

Experiment 3.—Subject 3, S. B. G. June 6, 1919, 5 to 6.30 p.m. Mucous membrane thermopile on left tonsil. Skin thermopile on forehead. Respirations 14 per minute. Mouth open; nostrils plugged. Thoracic and abdominal pneumographs. Room temperature 18.60–20.0°C.

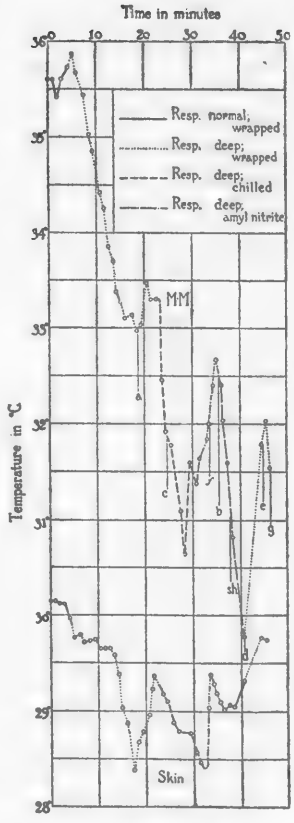
[7] Mudd and Grant,[1] p. 92.

In Text-fig. 5 the chilling and recovery curves proceed normally until 0:46, after which both skin and mucous membrane temperatures fall again, presumably on account of the subject's being inadequately wrapped. This curve is included in the composite although it makes less striking the hyperemia reaction to rewarming. The

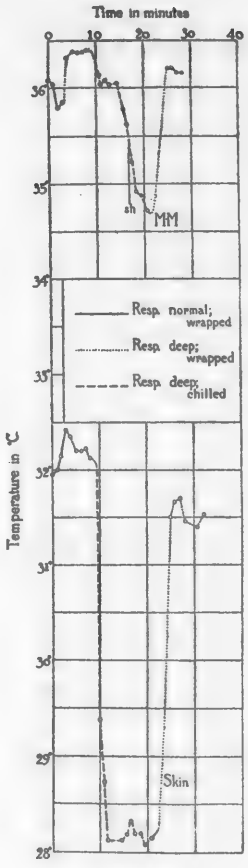


TEXT-FIG. 5. Reactions to chilling, rewarming, and amyl nitrite. Temperatures of skin and mucous membrane of faucial tonsil, Experiment 3. *a*, fan faster; *sh*, starts shivering; *NF*, face not flushed; *B*, second ampule of amyl nitrite inhaled; *F*, face flushed; *P*, still inhaling amyl nitrite; face paler; *C*, stops inhaling.

mucous membrane response to amyl nitrite is here a momentary fall of 0.23° in 1 minute, followed by a sharp rise of 0.90° in 2 minutes, with a more gradual return to normal in about 5 minutes. The predominating factor is evidently the local vasodilation.



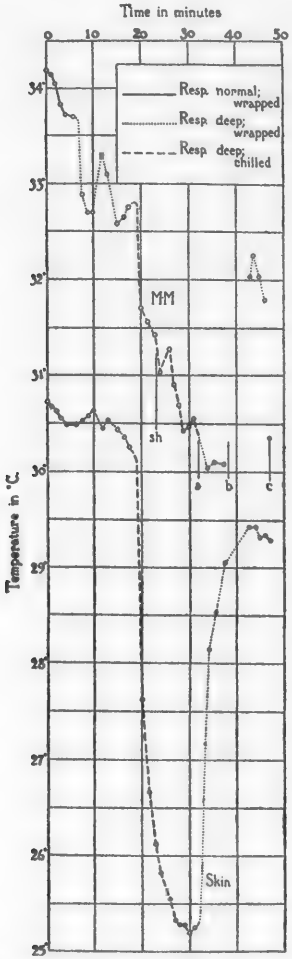
TEXT-FIG. 6.



TEXT-FIG. 7.

TEXT-FIG. 6. Reactions to chilling and amyl nitrite. Temperatures of skin and mucous membrane of faucial tonsil, Experiment 4. *a*, clears throat; *c*, coughed; *f*, face flushed; *b*, flush passing; stops inhaling amyl nitrite; *sh*, is shivering; *d*, clears throat; *e*, respiration too shallow; deepened; *g*, subject fainted.

TEXT-FIG. 7. Reactions to chilling and rewarming. Temperatures of skin and mucous membrane of faucial tonsil, Experiment 5. *sh*, is shivering.



TEXT-FIG. 8. Reaction to chilling. Temperatures of skin and mucous membrane of faucial tonsil, Experiment 6. Tonsil hypertrophic. *sh*, shivers; *a*, applicator holder seemed to be slipping; *b*, applicator off; reapplied; *c*, applicator tip off tonsil.

Experiment 4.—Subject 4, R. L. A. June 9, 1919, 10.56 to 11.46 a.m. Mucous membrane thermopile on left tonsil. Skin thermopile on forehead. Respirations 14 per minute. Mouth open; nostrils plugged. Thoracic and abdominal pneumographs. Room temperature 17.47–18.55°C.

In Text-fig. 6 the mucous membrane depression with chilling is great—3.52° in 18 minutes; the maximum skin fall, on the other hand, is only 0.88°, in 7.5 minutes, and is obliterated after amyl nitrite inhalation. Amyl nitrite, administered at 0:32.5 during chilling, produced a sharp rise in both mucous membrane and skin temperatures—1.02° in 2.5 minutes, and 0.89° in 1 minute, respectively. The recovery curves started up characteristically, but were interrupted by the subject's fainting.

Experiment 5.—Subject 3, S. B. G. June 11, 1919, 4.37 to 5.09 p.m. Mucous membrane thermopile on left tonsil. Skin thermopile on forehead. Respirations 10 per minute. Mouth breathing. Thoracic and abdominal pneumographs. Room temperature 19.65–20.12°C.

Text-fig. 7 shows characteristic chilling curves. Recovery is not quite complete either for skin or mucous membrane, perhaps due to inadequate wrapping. The tonsillar curve was cut short at 0:28 by the slipping of the thermopile applicator.

Experiment 6.—Subject 5, A. G. June 10, 1919, 11.30 a.m. to 12.17 p.m. Mucous membrane thermopile on right tonsil. Skin thermopile on forehead. Respirations 16 per minute. Mouth open; nostrils plugged. Thoracic and abdominal pneumographs. Room temperature 16.90–17.80°C.

The tonsils of this subject had been diagnosed in the Army and in the Washington University as hypertrophic. Text-fig. 8 shows characteristic chilling curves for both tonsil and skin, of large amplitude in each case. The applicator holder seemed to be slipping at 0:31.5, and satisfactory application was not thereafter obtained.

Discussion of Vasomotor Reactions to Chilling.[8]

The difference in recovery of blood supply upon rewarming after chilling exhibited by the palatine tonsils on the one hand, and the palate and pharynx on the other, is of interest, and suggests a corollary

[8] Compare Galeotti, G., *Riforma Med.*, 1920, xxxvi, 205; abstracted in *J. Am. Med. Assn.*, 1920, lxxiv, 1491.

hypothesis. It is well known that persons in robust health and inured to rigorous physical circumstances of living react better after chilling, as for instance by a sea bath, than the less vigorous. It seems possible that one factor in the hardening process of cold bathing, outdoor life, and so forth, with its incident heightened resistance to colds and sore throats, is a training of the vasomotor system in the direction of development of a quick reaction of hyperemia in the pharynx following chilling, such as has been shown to exist in the tonsils of the subjects of the present experiments.

We would reiterate in order to minimize any possibility of misunderstanding. We are concerned in our experiments with excessive chilling of the body surface, which, like overdosage of a useful drug, we believe may have ill effects. Certainly we would not encourage the unreasoning fear of slight drafts and exposure so often encountered. Good ventilation and circulating air in buildings, cold weather, and out-of-door living are needed for vigorous health; many people are unquestionably benefited by cold bathing. But excesses in this direction should also be avoided.

Autoinfections only are under consideration in the present paper. However, it should be clearly borne in mind that colds dependent primarily upon contagion from outside sources are probably of more frequent and widespread occurrence.[9]

Concurrent Bacteriological Studies.

Several instances during the experiments of 1918 seemed at least suggestive of experimental excitation of infection. The case of S. M. (Subject 6) in particular seemed to point to chilling as the exciting factor. The experiment was a blood temperature control in which he sat from 4 to 6.09 p.m., December 4, 1918, with closed mouth, the bulb of a thermometer beneath his tongue. He forced respiration from 4.12 to 6.09 p.m., and was chilled by a fan from 4.47 to 5.09 p.m. Shivering began at 4.48 p.m., and had become very severe by 4.52 p.m. The subject had not been aware of other recent exposure to cold or infection. By the morning of December

[9] For a discussion of this phase of the subject the reader is referred especially to Hill, L., The science of ventilation and open-air treatment. Part 1, *Gt. Britain Med. Research Com., Special Rep. Series, No. 32,* 1919.

5, nasopharyngeal stuffiness had developed sufficiently to cause the remark by a friend that he had a cold. The symptoms persisted 3 or 4 days.[10]

A study of the flora of the nose and throat during the experiments of the summer of 1919 seemed, then, to be well worth while.

Material and Method.—The medium employed was a 5 per cent rabbit blood meat infusion agar. Baked blood agar was also used in each instance as a special medium for *Bacillus influenzæ*. Sputum from each subject was injected into a mouse for typing pneumococcus. Cultures were taken from the nose through the anterior nares, from the tonsil, and from the posterior pharyngeal wall by means of separate swabs. Each swab was immersed in sterile broth and then applied both to a red and baked blood agar plate; the remaining area of the plate was inoculated by means of a platinum loop. Films were made directly from the same swab.

The cultures were incubated for 36 hours. The plates were then divided into eight segments, and every colony in two to four segments of the plate was counted and its nature determined. An attempt was made to count approximately the same total number of colonies daily, in order to detect any marked changes in the flora and particularly in the relative proportions of the bacteria present. On account of the difficulty of differentiating pneumococcus from *Streptococcus viridans* by morphology these two were put in the same group.

Results.—Four different subjects were used in this study. Two developed clinical sore throats; a third had some symptoms of malaise and headache; a fourth was unaffected. The results obtained with the nose cultures were entirely negative. Subjects 5 and 6 showed *Staphylococcus aureus*, Subjects 1 and 3 *Staphylococcus albus*. No attempt was made to sterilize the vestibule of the nose before swabbing. The flora obtained from the nose, as has been found by other investigators[11] was at all times exceedingly sparse, but the above organisms were always found to be present. They showed no changes with exposure. The results obtained from pharynx and tonsil in each instance are given in Tables I to III.

[10] Similarly we have noted what we believe to be an excitation of infection after, and presumably due to chilling in a number of carefully observed instances in our everyday experience.

[11] Thomson, St. C., Diseases of the nose and throat, New York, 1913, 7.

TABLE I.

Subject 6. Bacteriology of Tonsil and Pharynx.

Date.	Place cultured.	*S. viridans* and Pn. II.		*S. hæmolyticus.*		*B. influenzæ.*		Undetermined.		Remarks.
		No. of colonies counted.	Per cent of all colonies.	No. of colonies counted.	Per cent of all colonies.	No. of colonies counted.	Per cent of all colonies.	No. of colonies counted.	Per cent of all colonies.	
1919										
June 4	Left tonsil.	28	93	0	0	2	7	0	0	
	Pharynx.	36	100	0	0	0	0	0	0	
						Present in baked blood.				
" 5	Left tonsil.	43	100	0	0	" " " "		0	0	
	Pharynx.	31	94	0	0	" " " "		4	6	
" 6	Left tonsil.	35	100	0	0	" " " "		0	0	
	Pharynx.	24	86	0	0	" " " "		4	14	
" 7*	Left tonsil.	52	94	0	0	3	6	0	0	Subject of experiment. Application on left tonsil. Cultures after experiment.
	Pharynx.	40	87	0	0	4	13	0	0	
" 8	Left tonsil.	57	65	13	15	7	8	11	12	Subjective sore throat. Pharynx shows injection with white exudate; tonsillar ring injected.
	Pharynx.	26	56	14	31	6	13	0	0	
" 9	Left tonsil.	35	76	6	13	1	2	4	8	Very slight soreness. Throat no longer injected.
	Pharynx.	34	79	9	21	Present in baked blood.		0	0	
" 11	Left tonsil.	31	94	1	3	1	3	0	0	Throat normal.
	Pharynx.	30	91	3	9	Present in baked blood.		0	0	

* Experiment 7.

TABLE II.

Subject 1. Bacteriology of Tonsil and Pharynx.

Date.	Place cultured.	*S. viridans.*		*S. hæmolyticus.*		*B. influenzæ.*		*S. albus.*		Remarks.
		No. of colonies counted.	Per cent of all colonies.	No. of colonies counted.	Per cent of all colonies.	No. of colonies counted.	Per cent of all colonies.	No. of colonies counted.	Per cent of all colonies.	
1919										
June 10	Left tonsil.	18	42	4	9	9	21	12	28	
	Pharynx.	13	52	0	0	12	48	0	0	
" 11*	Left tonsil.	28	68	3	8	8	20	2	4	Subject of experiment. Application on left tonsil. Cultures after experiment.
	Pharynx.	24	72	0	0	6	18	3	9	
" 12	Left tonsil.	31	55	3	5	5	9	17	31	No after effects.
	Pharynx.	18	54	0	0	12	36	4	9	
" 16†	Left tonsil.	26	53	0	0	6	12	18	35	Subject of experiment. Application on soft palate. Cultures taken before experiment.
	Pharynx.	32	60	0	0	9	17	12	23	
" 17	Left tonsil.	14	45	2	7	8	26	7	22	Subject complains of general malaise; slight headache; no sore throat. Film from swab shows mostly Gram-negative bacilli with a few cocci.
	Pharynx.									
	Red blood.	0	0	0	0		100	0	0	
	Baked blood.	0	0	0	0		100	0	0	
" 19	Left tonsil.	19	56	2	6	5	15	8	24	
	Pharynx.	21	60	0	0	14	40	0	0	
" 20	Left tonsil.	21	50	3	7	7	17	11	26	
	Pharynx.	19	58	0	0	11	33	3	9	

* Experiment 1.

† Experiment 8.

TABLE III.

Subject 5. Bacteriology of Tonsil and Pharynx.

Date.	Place cultured.	*S. viridans.*		*M. catarrhalis.*		*S. aureus.*		*S. albus.*		Remarks.
		No. of colonies counted.	Per cent of all colonies.	No. of colonies counted.	Per cent of all colonies.	No. of colonies counted.	Per cent of all colonies.	No. of colonies counted.	Per cent of all colonies.	
1919										
June 7	Left tonsil.	29	85	0	0	0	0	5	15	
	Pharynx.	21	91	0	0	0	0	2	9	
" 8*	Left tonsil.	49	90	0	0	0	0	5	10	Subject of experiment. Application on left tonsil. Cultures after experiment.
	Pharynx.	27	75	0	0	2	6	7	19	
" 9	Left tonsil.	21	60	3	9	0	0	11	31	No symptoms.
	Pharynx.	31	57	15	27	1	2	7	14	
" 10†	Left tonsil.	27	52	4	8	2	4	19	36	Subject of experiment. Application on right tonsil. Cultures after experiment.
	Pharynx.	34	81	0	0	3	7	5	12	
" 11	Left tonsil.	34	85	1	2	0	0	5	13	
	Pharynx.	39	93	0	0	0	0	3	7	
" 12	Right tonsil.	30	73	0	0	4	10	7	17	
	Pharynx.	34	87	2	5	1	3	2	5	
" 13‡	Right tonsil.	40	88	2	5	0	0	3	7	Subject of experiment. Application on pharynx. Cultures after experiment.
	Pharynx.	27	70	7	19	0	0	4	11	
" 14	Right tonsil.	21	58	12	34	0	0	3	8	
	Pharynx.	15	45	4	11	11	39	2	5	
" 15										No cultures. Subject has soreness on swallowing.

June 16§	Right tonsil.	21	52	11	27	0	0	8	20	Subject of experiment. Application on pharynx. Marked congestion of tonsillar ring and pharynx.
	Pharynx.	29	60	15	31	0	0	4	9	
" 17	Right tonsil.	34	72	3	7	8	17	2	4	Throat injection milder. No soreness on swallowing.
	Pharynx.	27	75	6	16	3	8	0	0	
" 18‖	Right tonsil.	25	59	2	5	15	36	0	0	Subject of experiment. Application on soft palate. Cultures after experiment.
	Pharynx.	31	77	4	10	5	13	0	0	
" 19	Right tonsil.	28	54	15	28	9	18	0	0	Soreness on swallowing. Injection of tonsillar ring.
	Pharynx.	32	64	11	22	7	14	0	0	
" 20										No cultures. Sore throat disappeared.
" 21	Right tonsil.	35	83	0	0	4	10	3	7	No soreness. No injection.
	Pharynx.	31	93	1	3	0	0	1	3	
" 23	Right tonsil.	31	81	2	5	1	2	4	11	" " " "
	Pharynx.	27	81	1	3	2	6	3	9	

* Experiment 9.
† Experiment 6.
‡ Experiment 10.
§ Experiment 11.
‖ Experiment 12.

From the pharynx and tonsil of Subject 6 before the experiment there were cultured *Streptococcus viridans*, Pneumococcus Type II atypical, and *Bacillus influenzæ*. He was then subject of Experiment 7. Within 24 hours he had a clinical sore throat; coincident with this, there was a sudden appearance of *Streptococcus hæmolyticus* in the cultures from both tonsil and pharynx. During the days following, with the disappearance of the sore throat, the number of colonies of *Streptococcus hæmolyticus* fell off rapidly. The remaining bacteria showed no evident change.

Experiment 7.—Subject 6, S. M. June 7, 1919, 3.45 to 6 p.m. Mucous membrane thermopile on left tonsil. Respirations 14 per minute. Mouth open; nostrils plugged. Room temperature 18.10–19.60°C. 0:00 to 0:31.5. Wrapped; normal breathing; swallowed many times. 0:31.5 to 0:44. Wrapped; deep breathing. 0:44 to 0:55.5. Unwrapped; fan on back; deep breathing. 0:52. Shivers. 0:55.5. Coughs, chokes, applicator removed; blood flecks seen about terminals. 4.57 p.m. Experiment started again. 0:00 to 0:06. Wrapped; deep breathing. 0:06 to 0:13.5. Unwrapped; fan on; deep breathing; shivering; swallows many times. 0:13.5 to 0:33.5. Wrapped; deep breathing. 0:33.5. Conditions the same; amyl nitrite inhaled. 0:35 to 0:44. Wrapped; deep breathing; some coughing and swallowing. 0:44 to 0:53. Hot water bag around subject; wrapped; deep breathing.

On the following morning the feeling of soreness had practically left the traumatized tonsil, but the posterior wall of the oropharynx felt sore. On inspection a localized area of injection bearing a whitish exudate was seen on the posterior wall of the oropharynx. In culturing, the contact of the swab on this area was a little painful, and the culture yielded streptococci, as explained above. The tonsillar ring was injected, but the feeling of soreness on the tonsil entirely passed off during the day and contact of the swab in culturing was hardly felt. Thus the traumatized tonsil showed less evidence of being the site of an active infection than the posterior pharyngeal wall, which was thought not to have been directly traumatized, but of course we cannot exclude the possibility that the oropharynx was infected by hemolytic streptococci from the tonsillar crypts—so commonly a habitat for them—which were missed in the earlier cultures and were disseminated by the experimental trauma and swallowing.

In Subject 1 there were present *Streptococcus viridans*, *Bacillus influenzæ*, *Streptococcus hæmolyticus*, and *Staphylococcus albus*. Following Experiment 1 there were no noteworthy changes. The plate inoculated from the pharynx about 26 hours after Experiment 8, however, showed a pure culture of *Bacillus influenzæ;* the tonsillar

plate showed also a slight relative increase. The film from the pharynx showed practically all Gram-negative bacilli with an occasional coccus. The subject had no sore throat, but complained of general malaise, slight headache, and some chilly sensations.[12] The pharynx culture made 96 hours after Experiment 8 was practically the same as before the experiment, *Streptococcus viridans* and *Staphylococcus albus* appearing as before.

Experiment 8.[13]—Subject 1, W. G. E. June 16, 1919, 3.20 to 4.07 p.m. Mucous membrane thermopile on anterior half of soft palate, left side. Respirations 16 per minute. Mouth open; nostrils plugged. Room temperature 20.22–20.90°C. 0:00 to 0:05.5. Wrapped; normal breathing. 0:05.5 to 0:11. Wrapped; deep breathing. 0:11 to 0:32. Unwrapped; fan on back; deep breathing. 0:32 to 0:47. Wrapped; deep breathing.

With the applicator resting against the anterior soft palate there is no reason for supposing trauma to the tonsils and pharynx. Organisms could hardly have been introduced from outside by the applicator, for this, with the thermopile terminals attached, was freshly coated with shellac in alcoholic solution before being adjusted for an experiment.

Subject 5 was the subject of five experiments, June 8, 10, 13, 16, and 18. On June 15 he noted a soreness on swallowing, and on June 16 the entire posterior pharynx and tonsillar ring were distinctly injected. The experiment of June 16 produced no sudden increase in symptoms; on June 17 the sore throat had practically disappeared. On June 19, 36 hours following an experiment, the subject again developed a soreness on swallowing, with congestion of the posterior pharynx and tonsillar ring. On June 20 the symptoms had subsided. On June 21 the wares no longer any injection or soreness.

This subject had present in his throat *Streptococcus viridans*, *Staphylococcus aureus*, and *Staphylococcus albus*. 24 hours following the first experiment there was noted for the first time the appearance of *Micrococcus catarrhalis*. Subsequently there appeared to be a certain degree of correlation between the appearances of sore throat and the rises in relative numbers of *Micrococcus catarrhalis* colonies. No other change in the bacterial flora was apparent.

[12] Compare the effect of infecting monkeys with *B. influenzæ* (Blake, F. G., and Cecil, R. L., *J. Am. Med. Assn.*, 1920, lxxiv, 170).

[13] For Experiment 1 see p. 93.

Experiment 9.—Subject 5, A. G. June 8, 1919, 3.38 to 5.06 p.m. Mucous membrane applicator on left tonsil. Respirations 10 per minute. Mouth open; nostrils plugged. Room temperature 18.1–19.45°C. 0:00 to 0:06. Wrapped; normal breathing. 0:06 to 0:15. Wrapped; deep breathing. 0:15 to 0:43. Unwrapped; fan on back; deep breathing; some swallowing, coughing, and clearing of throat; after 0:17, shivering. 0:43 to 1:20. Wrapped; deep respiration. 1:07. Inhales amyl nitrite. 1:20 to 1:28. Hot water pad to back; deep respiration.

The possibility that trauma to the tonsil was responsible for the appearance of *Micrococcus catarrhalis* after this experiment cannot be excluded.

Experiment 10.[14]—Subject 5, A. G. June 13, 1919, 3 to 3.46 p.m. Mucous membrane applicator on posterior wall of oropharynx. Respirations 16 per minute. Mouth open; nostrils plugged. Room temperature 18.45–19.05°C. 0:00 to 0:04.5. Wrapped; normal breathing. 0:04.5 to 0:15. Wrapped; deep breathing. 0:15 to 0:26. Unwrapped; fan on back; deep breathing; coughed; cleared throat; contraction of pharyngeal muscles; after 0:22, shivers. 0:26 to 0:46. Wrapped; deep breathing; coughed and cleared throat several times; pharynx appeared normal.

Experiment 11.—Subject 5, A. G. June 16, 1919, 10.30 to 11.30 a.m. Mucous membrane applicator on posterior pharyngeal wall. Respirations 14 per minute. Mouth open; nostrils plugged. Room temperature 18.90–19.80°C. 0:00 to 0:04.5. Wrapped; normal breathing; contractions of pharyngeal muscles. 0:04.5 to 0:23.5. Wrapped; deep breathing. 0:23.5 to 0:32. Unwrapped; fan on back; deep breathing. 0:32 to 1:00. Wrapped; deep breathing; contractions of pharyngeal muscles.

Experiment 12.—Subject 5, A. G. June 18, 1919, 10.20 to 11.12 a.m. Mucous membrane applicator on soft palate, middle part. Respirations 14 per minute. Mouth open; nostrils plugged. Room temperature 18.75–19.55°C. 0:00 to 0:05.5. Wrapped; normal breathing. 0:05.5 to 0:15.5. Wrapped; deep breathing. 0:15.5 to 0:27. Unwrapped; fan on back; deep breathing. 0:27 to 0:52. Wrapped; deep breathing.

In Subject 3 there were present in the throat *Streptococcus viridans*, Pneumococcus Type IV, and *Staphylococcus albus*. Cultures were made daily from June 4 to 19; he was the subject of an experiment on June 6, 9, 14, and 17. There was practically no change in the bacterial flora of this subject throughout the entire period studied. Neither did subjective or objective signs of sore throat or cold develop. Frequently the cultures from the pharynx were almost sterile, there being from three to ten colonies over the entire plate.

[14] For Experiment 6 see p. 99.

Discussion of Bacteriological Results.

Streptococcus viridans was found in all four of the individuals studied, *Bacillus influenzæ* in two, pneumococcus in two, and *Micrococcus catarrhalis* in one subject.

In Subject 6 the increased number of *Streptococcus hæmolyticus* was definitely synchronous with the presence of a sore throat. There appears to have been a correlation between the high *Micrococcus catarrhalis* counts and the presence of sore throats in Subject 5. The pure culture of *Bacillus influenzæ* in the pharynx of Subject 1 was not coincident with sore throat but with malaise, slight chilliness, and headache.

These results in no sense prove, however, that the sore throats were caused by the increased number of bacteria cultured from the mucous membranes, or that the apparent increase of microorganisms was caused by the ischemia of the mucous membranes incident upon chilling of the body surface. The method is subject to so many sources of error, and the amount of data thus far obtained is so small, that we do not feel justified in drawing any conclusions. To attribute the apparent proliferation of pathogenic microorganisms to the effect of chilling would seem to be in harmony with the great wealth of clinical and common observation which points to excessive chilling, under proper circumstances, as an efficient excitant of infection of the mucous membranes by their indigenous pathogenic bacteria. Although it is possible that the apparent proliferation was due to the local ischemia incident upon chilling, the inaccuracy of the bacteriological method and the insufficient data make it impossible to assume that this is so. The effect of trauma by the thermopiles, the possibility of transient changes in the flora of the mucous membranes caused by swallowing, gagging, or other muscular activity in the pharynx pressing a plug of bacteria from the tonsillar crypts, the fact that the subject's mouth was held open throughout the experiments, with the accompanying accumulation of mucus on the membranes, the errors necessarily introduced in each stage of making the cultures, and the inaccuracy of any method depending upon swab cultures, all tend to confuse the results. We present the data given above as a contribution to the etiology of upper respiratory infections, and not with the idea that the study is in any sense complete in itself.

TABLE IV.

Observations on Concentration and Reaction of Urine.

Experiment No.	Increase in respiration.	Deep respiration.	Deep respiration; chilled.	Deep respiration.	Reaction to litmus.		Color.		Clarity.		Decrease in acidity in amount of 0.1 N NaOH neutralized by 10 cc. of urine.
					Before experiment.	After experiment.	Before experiment.	After experiment.	Before experiment.	After experiment.	
	per cent	*min.*	*min.*	*min.*							*cc.*
9*	77.9	9	28	45					Clear.	Cloud of phosphates.	
4*	88.9	18.5	17.5	6		Neutral.	Dark yellow.	Light yellow.			
13*		17.5	19.5	21	Acid.	Acid.	Yellow.	Very slightly lighter.			
6	75.4	12	13.5	15	"	Alkaline.	"	Light yellow.	Clear.	Phosphates.	4.25 (titrated next day).
1	42.7	6	5.5	42.5	"	"	"	" "	"		1.76
14	45.4	11.75	22	17	"	Neutral to alkaline.	"	Slightly lighter yellow.	"	Cloudy.	1.24
15											
10	69.2	10.5	11	20	"	Alkaline.					2.54
16	54	7.5	22	20	"	"					2.16
11	30.1	19	8.5	28	"	"					4.90
8	30.1	5	21	15.5	"	Acid.					0.00
17	44.6	6	16	19.5	"	"					2.28
12	52.3	10	11.5	28	Neutral.	Alkaline.					0.95
Average.	49.3	9.75	14.56	22.83							2.23

* Not included in average.

Observations on Urine.

It was thought that the chilling in the experiments might produce sufficient renal congestion to give albuminuria. This, however, was not the case. In fourteen experiments in which urine specimens were taken before and after the experiment the results were in all instances negative. The room temperature in these experiments varied from 16.9–20.9°C.; the time of exposure, unwrapped, to the draft of the electric fan was from 5.5 to 28 minutes, the average 16.75 minutes. Severe shivering was often produced. In twelve of the experiments urinalysis for sugar was done, also with negative results in all.

In all of five experiments in which the color was noted it was lighter after than before the experiment, denoting the secretion of a more dilute urine during the experiment.

The most interesting change in the urine produced by the experimental conditions was a decrease in its acidity. Specimens were titrated with phenolphthalein against 0.1 N sodium hydroxide in nine experiments; in eight a decrease in acidity after the experiment was noted, amounting to the equivalent of from 0.95 to 4.90 cc. of 0.1 N sodium hydroxide per 10 cc. of urine; the average decrease was 2.23 cc. This lowered titer was doubtless due in part to dilution of the urine, but in part also represented a true fall in hydrogen ion concentration, as is shown by the fact that a change in the urinary reaction demonstrable with litmus paper was noted in seven out of ten experiments. The mechanism of the experimental alteration in the reaction of the urine has been further analyzed by Grant and Goldman;[15] the decreased acidity has been found to be referable to the forced respiration rather than to the chilling.

The results of the observations on concentration and reaction of urine are given in Table IV.

SUMMARY.

Improvements are described in the method of following temperature changes, and thus alterations in vasomotor tone, in exposed mucous membranes. Invention of an applicator holder, by means of which

[15] Unpublished investigation.

more sure and stable apposition of the thermopile terminals to the mucous surface may be effected has been the chief advance. Minor improvements have been the use of a saliva ejector and of better calibration technique.

The palatine tonsils, like the palate, pharynx, and skin, react to chilling of the body surface with reflex vasoconstriction and ischemia. On rewarming the subject the tonsils quickly more than recover their former blood supply, actually becoming hyperemic; the skin returns to about its control condition; the pharynx and palate remain somewhat ischemic.

The hypothesis is advanced that one factor in the beneficial hardening effect of cold bathing and outdoor living, with its incident heightened immunity to respiratory infection, may be the training of the vasomotor system in the direction of development in the pharynx of a reaction of hyperemia following chilling, similar to that observed in the tonsils of the present subjects.

With inhalation of amyl nitrite, the skin temperature has always shown a sharp transient rise. The mucous membrane, if relatively ischemic, responds by a rise corresponding to the skin flush. If already hyperemic, local vasodilation in the mucous membrane with amyl nitrite is more than counterbalanced by the lowering of the general blood pressure, and the temperature falls.

The flora of the pharynx and tonsils, studied by the unsatisfactory method described, showed, in several instances, after experimentation changes apparently due to proliferation of one of the microorganisms already present. In one case *Streptococcus hæmolyticus*, in one *Micrococcus catarrhalis*, and in a third *Bacillus influenzæ* was the organism showing a relative increase in numbers. The first two instances were associated with sore throat, the third with slight constitutional symptoms.

The chilling in the experiments in no instance produced albuminuria or glycosuria, although a more dilute urine was apparently excreted during the experiments. A fall in hydrogen ion concentration, referable to the forced respiration, was noted.

It is a pleasure to thank Professor Joseph Erlanger of the Department of Physiology for his many courtesies.

HOMEOTRANSPLANTATION AND AUTOTRANSPLANTATION OF THE SPLEEN IN RABBITS.

III. Further Data on Growth, Permanence, Effect of Age, and Partial or Complete Removal of the Spleen.

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(Received for publication, February 27, 1920.)

We have been unable to find any references in the literature to the transplantation of fragments of spleen to parts of the body widely separated from the normal neurovascular field of this organ in addition to those referred to in 1917. At that time[1, 2] we reviewed the literature and reported our first experiments with spleen homeografts and autografts in fifteen rabbits. The present paper includes the data of further experiments with homeografts and with autografts, together with certain general physiological reactions relative to the spleen which this study has emphasized.

Method.

The method consists of transferring small fragments of the spleen roughly 2 by 2 mm. to the subcutaneous tissues of the abdomen under strict aseptic precautions. The tissue to be transplanted was kept in isotonic salt solution at temperatures varying between 36° and 39°C. Sometimes the tissue was transplanted within a few minutes after removal, while in others it was kept in salt solution as long as 3 hours. The fragment to be transplanted was rinsed in the salt solution, but no other attempt to remove contained blood was made.

[1] Manley, O. T., and Marine, D., The transplantation of splenic tissue into the subcutaneous fascia of the abdomen in rabbits, *J. Exp. Med.*, 1917, xxv, 619.

[2] Marine, D., and Manley, O. T., Influence of age on the permanence of subcutaneous autografts of the spleen in rabbits, *Proc. Soc. Exp. Biol. and Med.*, 1916–17, xiv, 123.

After making a transverse abdominal skin incision approximately 2 cm. in length, usually one on each side of the umbilicus, the subcutaneous fascia was lifted with fine forceps and an area free from blood vessels was punctured with a cataract knife, the tissue introduced, and the fascial opening closed with a ligature. The skin incision was sutured and sealed with celloidin. All operative procedures were carried out under ether anesthesia. All subsequent examinations were direct (autopsy or operation). If the examination was made by operation, ether anesthesia was used, and with aseptic precautions the skin was incised, the graft or its site was exposed, measurements were taken, the graft was removed for microscopic examination or left *in situ*, and the skin closed with suture and sealed with celloidin. The intervals between examinations are given in the tables. This technique has been employed in all our transplantation work with ductless gland tissues.

Both homeografts and autografts were studied in relation to taking and growth or regeneration, in relation to age of the animals, and in relation to partial and complete removal of the spleen and to permanence of the grafts.

Homeotransplantation.

The data of the experiments are given in Table I. Only the rabbits which had not been subjected to previous homeografts of any tissue are included in the table. This was done to avoid the untoward effect of active immunity which such grafts produce and to render conditions as favorable as possible for taking and growth. Notwithstanding this important precaution not a single graft was found active after the 30 day period, a period arbitrarily chosen, but somewhat longer than the time necessary for the full development and destructive effect of the active immunity. The experiments were not studied from the standpoint of determining how rapidly the grafts were destroyed, though by comparison they show very clearly that the destruction of spleen is much more rapid than that of thyroid tissue transplanted under the same conditions. Thus in a large number of routine homeotransplants of thyroid we have obtained about 10 per cent active at the end of 30 days, though ultimately (in the course of

60 to 90 days) these also might be destroyed. These observations indicate that spleen tissue produces a high degree of immunity more quickly than does thyroid.

The well established favorable factor of blood relation in the transplantation of certain tissues was also utilized in the hope that grafts might survive beyond the 30 day period, but with spleen we were unsuccessful. The antigenic power of spleen, then, is very high and on a level with that of other hematopoietic tissues. In a few instances in which the grafts were examined at the 7th, 8th, and up to the 16th day, active splenic tissue, well established blood supply, and evidence of growth were found. This is observed generally with homeografted tissues. Many literature reports of positive homeografts of tissues are misleading because they have emphasized or reported only this early stage. Effective immunity to foreign proteins in general does not become manifest until the 8th to 10th day, as shown by studies on anaphylaxis. Within this period homeografts usually take and often show growth, while after this period rapid destruction takes place. Different tissues show different rates of destruction. In our experience lymphoid (lymph gland and thymus) and splenic tissues undergo destruction more rapidly than thyroid, and thyroid cells more rapidly than cells rich in lipoids, as adrenal cortex and interstitial cells of the ovary and testis. Associated with this destruction there is the well known infiltration or invasion of the graft with lymphoid cells. Much importance has been assigned to this phenomenon and some authors, notably Murphy and his coworkers,[3] have claimed for it a primary importance in the death and destruction of homeografts. We cannot subscribe to this view. We believe that the primary injury is due to the action of antibodies and that the lymphoid cells are attracted to the site secondarily, and in response to a special low grade irritation which injured cells set up. Loeb[4] and Sittenfield[5] also have expressed the view that lymphocytes are not primarily concerned in the destruction of foreign proteins.

[3] Murphy, Jas. B., and Morton, J. J., The lymphocyte in natural and induced resistance to transplanted cancer. II, *J. Exp. Med.*, 1915, xxii, 204.

[4] Loeb, Leo, Multiple transplantations of the thyroid and the lymphocytic reaction, *J. Med. Research*, 1918–19, xxxix, 71.

[5] Sittenfield, M. J., The significance of the lymphocyte in immunity to cancer, *J. Cancer Research*, 1917, ii, 151.

TABLE I.

Homeotransplantation of Spleen.

Rabbit No.	Series No.	Age.	Sex.	Date. Splenectomy.	Date transplanted. Spleen used.		First examination.	Final examination.	Additional data.
					R.*	L.			
		days		*1917*	*1917*	*1917*			
1	4–46	26	M.	Jan. 18 Complete.		Jan. 18 3–155		L. 54–	Jan. 18. L. adrenalectomy. Thyroids and thymus enlarged.
2	4–48	33	F.	Jan. 25 Complete.		Jan. 25 3–157	L. 47±†	" 132–	Thymus atrophic. Jan. 25. L. adrenalectomy; homeograft of adrenal.
3	4–52	40	M.	Feb. 1 Complete.	Feb. 1 3–159		R. 40–	R. 125–	Feb. 1. L. adrenalectomy. Thymus present; thyroid large.
4	4–54	47	"	Jan. 30 Complete.	Jan. 30 3–161			" 42–	Jan. 30. L. adrenalectomy; homeograft of adrenal. Thymus large; thyroid normal.
5	4–64	54	F.	Feb. 6 Complete.	Feb. 6 3–163		R. 35–	" 121–	Feb. 6. L. adrenalectomy. Thymus atrophic; thyroid normal.
6	4–74	61	"	Feb. 13 Complete.	Feb. 13 3–165		" 58–	" 114–	Feb. 13. L. adrenalectomy; homeograft of adrenal. Thymus large; thyroid normal.
7	4–94	90	M.	Mar. 22 Complete.		June 7 3–184		L. 98–	Mar. 22. L. adrenalectomy. Thymus cellular; thyroid normal.
				1915		*1915*			
8	4–17	123	"	Dec. 3 Complete.		Dec. 16 1–320		" 67–	Spleen was enlarged; thymus small; adrenals small. Dec. 3. Thyroidectomy.
				1917		*1917*			
9	4–78	168	"	Feb. 15 Complete.		Feb. 15 3–169		" 56–	Feb. 15. L. adrenalectomy. Chronic nephritis; thymus atrophic; thyroid and parathyroids enlarged.

				1915		*1915*			
10	4–2	Adult.	M.	Aug. 26 Complete.		Nov. 29 1–313		L. 15—	Nov. 29. Thyroidectomy.
11	4–3	"	F.	Aug. 26 Complete.		Dec. 16 1–320		" 65—	" 29. "
					1915				
12	4–4	"	M.	Nov. 5 Complete.	Dec. 16 1–320	Nov. 29 1–313		R. 67— L. 17—	" 29. "
13	4–5	"	"	Nov. 5 Complete.	Dec. 18 1–321			R. 63—	Dec. 2. "
14	4–6	"	F.	May 11 Complete.		Dec. 2 1–315 Dec. 16 1–320		L. 14±(Dec. 2). " 65—(" 16).	" 2. "
15	4–7	"	M.	Nov. 5 Complete.		Dec. 16 1–320		L. 65—	" 2. "
16	4–8	"	"	Nov. 5 Complete.		Dec. 16 1–320		" 67—	" 3. "
17	4–9	"	F.	Nov. 5 Complete.		Dec. 16 1–320		" 119—	" 3. "
				1916		*1916*			
18	4–42	"	"	Dec. 12 Complete.		Dec. 12 3–151		" 43—	" 12. " L. adrenalectomy.
					1917				
19	4–44	Old adult.	M.	Dec. 19 Complete.	Feb. 20 1–411			R. 23—	Dec. 12, 1916. Thyroidectomy; L. adrenalectomy.
				1917		*1917*			
20	4–93	52	F.	Mar. 20 ½ cm. Apr. 17 1 cm.		Apr. 17 3–179		L. 52—	Mar. 20. L. adrenalectomy. Thymus atrophic; thyroid normal.

* R. indicates right; L., left.

† The condition of the transplants at the different examinations is expressed by plus or minus signs instead of the actual measurements obtained in order to facilitate comparison. The figures express the intervals in days.

TABLE I—*Continued.*

Rabbit No.	Series No.	Age.	Sex.	Date. Splenectomy.	Date transplanted. Spleen used. R.*	Date transplanted. Spleen used. L.	First examination.	Final examination.	Additional data.
		days		*1917*	*1917*	*1917*			
21	4–104	80	M.	Apr. 17 ½ cm.		Apr. 17 3–179	L. 53—	L. 163—	All organs intact; same litter as Nos. 4–105 and 4–106; thyroid slightly enlarged; thymus present.
22	4–105	80	"	Apr. 17 1 cm.		Apr. 17 3–179		" 53—	All organs intact.
23	4–106	80	"	Apr. 17 1 cm.		Apr. 17 3–179	L. 53—	" 163—	" " " thymus small.
24	4–108	80	"	Apr. 17 ⅔ cm.		Apr. 17 3–192		" 52—	" " "
25	4–98	104	F.	Apr. 10 ½ of spleen.		Apr. 10 3–177	L. 58±	" 169—	Thyroid lobes large, vascular; thymus normal.
26	4–101	60	M.		Apr. 10 3–177			R. 56—	June 26, 1916. R. adrenalectomy. Oct. 10. Gonadectomy. Mar. 13, 1917. Thyroidectomy. Spleen small; thymus very large.
		mos.							
27	4–65	4	"			Feb. 6 3–163		L. 23—	Feb. 6. Thyroidectomy; much enlarged. H_2SO_4.
28	4–49	4	F.			Jan. 25 3–158		" 21—	Parathyroids very large; thymus large. Jan. 25. Thyroidectomy; H_2SO_4.
29	4–50	4	"			Jan. 25 3–157		" 40—	" 25. " H_2SO_4. Spleen small; parathyroids very large.
30	4–55	4	"		Jan. 30 3–162		R. 14++	R. 35—	Jan. 30. Thyroidectomy; H_2SO_4. Spleen enlarged; parathyroids very active.

31	4–56	4	F.		Jan. 30 3–162			R. 30–	Jan. 30. Thyroidectomy; H_2SO_4. Spleen normal; thymus active.
32	4–57	4	"		Jan. 30 3–162			" 30–	Jan. 30. Thyroidectomy; H_2SO_4. Adrenals and spleen normal; thymus atrophic.
33	4–58	4	M.		Jan. 30 3–162			" 30–	Jan. 30. Thyroidectomy; KOH.
34	4–59	4	"		Feb. 1 3–159			" 28–	Feb. 1. " H_2SO_4. Spleen and thymus normal.
35	4–60	4	"		Feb. 6 3–159			" 28–	Feb. 6. Thyroidectomy; H_2SO_4 and KOH. Spleen small; thymus very large.
36	4–61	4	F.			Feb. 6 3–163		L. 23+	Feb. 6. Thyroidectomy; H_2SO_4. Thymus atrophic.
37	4–76	5	M.			Feb. 13 3–166	L. 16+	" 42–	Feb. 13. Thyroidectomy; KOH.
38	4–62	5	F.			Feb. 6 3–163		" 23–	" 6. " KOH.
39	4–75	Adult.	"			Feb. 13 3–165	L. 16+	" 42–	" 13. " H_2SO_4 and KOH.
40	4–81	"	"			Feb. 15 3–169		" 8+	Jan. 23. Thyroidectomy.
41	4–82	"	"		Feb. 15 3–174			R. 42–	" 23. "
42	4–99	"	M.		Apr. 10 3–177			" 7+	Sept. 28, 1916. R. adrenalectomy. Jan. 16, 1917. L. adrenalectomy. Mar. 3. Thyroidectomy; H_2SO_4. Thymus atrophic; spleen small.
43	4–100	"	"		Apr. 10 3–177			" 58–	Sept. 22, 1916. R. adrenalectomy. Jan. 11, 1917. L. adrenalectomy. Mar. 13. Thyroidectomy. Thymus atrophic; spleen small.

TABLE I—*Concluded.*

Rabbit No.	Series No.	Age.	Sex.	Date. Splenectomy.	Date transplanted. Spleen used. R.*	Date transplanted. Spleen used. L.	First examination	Final examination.	Additional data.
					1917				
44	4–102	Adult.	M.		Apr. 10 3–177			R. 7+	May 25, 1916. R. adrenalectomy. June 1. L. adrenalectomy. Dec. 7. Thyroidectomy; gonadectomy. Spleen small; thymus atrophic.
45	4–109	"	"		Apr. 10 3–177			" 58—	Sept. 26, 1916. R. adrenalectomy. Jan. 11, 1917. L. adrenalectomy. Mar. 13. Thyroidectomy. Thymus large; spleen normal; L. adrenal much enlarged.
46	4–40	"	"		Jan. 25 3–158			" 26—	Dec. 26, 1916. Thyroidectomy; H_2SO_4.
						1916			
47	4–21	"	"			Dec. 19 3–154		L. 34—	Mar. 11, 1916. R. adrenalectomy. Dec. 19. L. adrenalectomy. Jan. 9, 1917. Gonadectomy. Thyroid normal; thymus very large.
48	4–39	"	"		Jan. 25 3–158			R. 26—	Jan. 2. Thyroidectomy.
49	4–43	"	"		Jan. 25 3–158			" 26—	" 2. " Previous homeografts.
						1917			
50	4–87	Old adult.	F.			Feb. 27 3–167		L. 23—	Jan. 22. Thyroidectomy. Many previous homeografts.

Effect of Age.—This factor has no appreciable influence. In this series rabbits of known age and parentage, varying from 26 to 168 days, as well as a large number of adults of different but known ages, were used. In no instance did the graft survive the 30 day period. Variations in the rate of destruction depending on age must be expected, but their detection in the case of spleen would require frequent examinations within the 30 day period. Further attempts were made to determine whether splenectomy, partial or complete, had any effect. No noteworthy difference in the outcome even in young rabbits was detected whether the spleen was intact, or partially or completely removed at the time of, before, or after transplantation. The experiments do not eliminate the possibility of an advantage accruing to the transplant from induced splenic insufficiency, but indicate that if splenectomy is of aid it must be looked for by frequent examinations within the first 30 days, since by that time the developed immunity has destroyed any possible evidence.

Thyroidectomy and partial or complete adrenalectomy combined or separate likewise have no noteworthy effect on delaying destruction of the grafts and, therefore, probably on the degree of immunity developed. In this connection it may be pointed out that Gates[6] was unable to detect any noteworthy difference in the degree of immunity to sheep erythrocytes and typhoid bacilli obtained in guinea pigs with and without partial removal of the adrenal glands. We have found[7] that thyroidectomy and splenectomy alone or combined have no marked effect on the antibody formation following injection of sheep erythrocytes in rabbits. Bullock and Rohdenburg[8] find that splenectomy has no influence on the immunity to transplanted tumors.

[6] Gates, F. L., Antibody production after partial adrenalectomy in guinea pigs, *J. Exp. Med.*, 1918, xxvii, 725.

[7] Unpublished results.

[8] Bullock, F. D., and Rohdenburg, G. L., Splenectomy exerts no appreciable influence upon immunity against transplanted tumors, *J. Cancer Research*, 1917, ii, 465.

Autotransplantation.

The data concerning these experiments are given in Tables II and III. Usually two fragments were transplanted, one to the right and the other to the left of the umbilicus, and designated as right and left transplants. In the six experiments reported in 1917 one failed and in the present series one failed. This was due to infection. Failure of autografts to take always indicates some gross technical error. Some of these rabbits had been used for homeotransplantation of other tissues—adrenal, thyroid, and sex glands. Previous or coincident homeografts have no demonstrable effect on the taking or growth of autografts. The subsequent course of these autografts was followed and it was found to be modified by several factors, the most striking of which are age and partial or total removal of the spleen.

Effect of Age.—The experiments have been arranged in Tables II and III according to the age at the time of transplantation. The youngest rabbit used was 26 days old and the oldest whose age was definitely known was 320 days; one other is listed as 586+ days because it had been in the laboratory for this length of time before being utilized for this purpose. A maximum of three examinations is given in the tables, although in several instances five, six, and more examinations have been made. A consideration of the series as a whole shows a distinct decrease in the amount of growth of the transplants as one passes from the youngest to the oldest rabbits of the series, although other factors remained as constant as possible. The greatest and most rapid growth of transplants occurred in the youngest rabbits, also the least growth in the oldest. While this decrease with age in the rate and amount of growth is gradual, the differences become striking about the 4th to 5th month. In our series the first instance of failure to obtain marked growth was in a rabbit 132 days old. The remaining ten rabbits, 167 or more days old at the time of transplantation, failed to show marked growth. This change in the rate of growth corresponds roughly with the time of sexual maturity or early adult life. There is a suggestion that in adult rabbits transplants that in the 1st month showed some growth tended to involute or undergo atrophy later. This tendency to atrophy or

involution was not seen in young rabbits with transplants of similar duration. As suggested in a previous note[2] two factors (possibly others) may be considered. The first is that growth of the transplants is a part of the normal growth of the animal and ceases when physical growth is complete. This possibility is disproved by the fact that such transplants fail to show marked growth even in the young if the spleen is not removed. The second factor would presuppose that the value of the spleen to the organism decreases with age and that after adult life whatever functions the organ normally has may be assumed by other tissues, possibly lymph glands and bone marrow. This view is supported by the results obtained by the removal of the spleen which follow.

Effect of Partial and Complete Removal of the Spleen on Autografts. —The data of these fifteen experiments are given in Table III. The amount of spleen removed and the dates are given in the table. Usually less than 0.5 cm. of the anterior portion was removed at the time of transplantation.[9] The youngest rabbit with partial removal of spleen and transplantation was 52 days old and the oldest 124 days. The same differences in the growth of transplants, dependent upon age and independent of amount of spleen removed, are noted as in Table II. Thus in No. 1, a 52 day old rabbit in which one-fourth of the spleen was removed, the transplants were ++ at 80 days and +++ at 177 days, while in No. 15, a 124 day old rabbit in which one-eighth of the spleen was removed, no growth took place in 132 days. Between these extremes there is the same evidence of gradation of growth dependent upon age as shown in Table II. There is no instance of a very young rabbit with a minimum amount of spleen removed. Such experiments would show whether in the absence of splenic deficiency marked growth could occur. This question, however, is clearly answered in the negative for 52 day old rabbits by Experiments 1, 2, and 3, in which all were from the same litter. Two had about one-fourth of the spleen removed at the time of transplantation and at the first examinations 80 and 52 days later both were ++, while the third, which had only one-eighth of the

[9] There are considerable variations in the size of the spleen in apparently healthy rabbits, just as in other animals, which cannot be accounted for with our present knowledge of physiology and pathology.

TABLE II.

Autotransplantation of Spleen (Complete Removal of Spleen).

Rabbit No.	Series No.	Age.	Weight.	Sex.	Date of splenectomy (complete).	Date transplanted.	First examination.	Second examination.	Final examination.	Additional data.
		days	*gm.*		*1917*	*1917*				
1	4–45	26	330	M.	Jan. 18	Jan. 18	R. 54+++ L. 54+++	R. 145+++ L. 145+++	R. 278+++ L. 278+++	Jan. 18. L. adrenalectomy. May. 1. Thyroidectomy.
2	4–46	26	315	"	" 18	" 18	R. 54+++	R. 139+++	R. 141+++	Jan 18. L. adrenalectomy.
3	4–47	33	575	"	" 25	" 25	" 47++ L. 47++	" 96+++ L. 96+++	" 230+++ L. 230+++	" 25. " " H_2SO_4. May 1. Thyroidectomy.
4	4–48	33	505	F.	" 25	" 25	R. 47++	R. 132+++	R. 134+++	Jan. 25. L. adrenalectomy.
5	4–51	40	330	"	Feb. 1	Feb. 1	" 40++ L. 40++	" 110++++ L. 110+++	" 224++ L. 224++	Feb. 1. " " H_2SO_4. May 1. Thyroidectomy; transplants slightly decreased.
6	4–52	40	415	M.	" 1	" 1	" 40++	" 125++	" 232+++	Feb. 1. L. adrenalectomy. Thyroids markedly enlarged at autopsy.
7	4–53	47	405	F.	Jan. 30	Jan. 30	R. 42++ L. 42++	R. 226++++ L. 226++++	R. 384++++ L. 384++++	Jan. 30. L. adrenalectomy; H_2SO_4. May 1. Thyroidectomy.
8	4–63	54	410	"	Feb. 6	Feb. 6	R. 35++ L. 35++	R. 84+++ L. 84+++	R. 121+++ L. 121+++	Feb. 6. L. adrenalectomy; KOH. May 1. Thyroidectomy.
9	4–64	54	310	"	" 6	" 6	" 35++	" 121++	" 133++	Feb. 6. L. adrenalectomy. Thyroids small.

10	4–73	61	555	F.	Feb. 13	Feb. 13	R. 58++++ L. 58++++	R. 77++++ L. 77++++	R. 119++++ L. 119++++	Feb. 13. L. adrenalectomy; KOH. May 1. Thyroidectomy.
11	4–94	61	595	"	" 13	" 13	" 58+++	" 114+++	" 115+++	Feb. 13. L. adrenalectomy. Thyroid normal.
					1916	*1916*				
12	4–38	66	1,275	M.	Dec. 7	Dec. 7	" 36+	" 82++	" 183++	Dec. 6. L. adrenalectomy; phosphoric acid.
13	4–37	66	875	F.	" 7	" 7	" 36+	" 56++	" 82+++	Dec. 6. L. adrenalectomy; phosphoric acid.
14	4–36	66	1,225	M.	" 7	" 7	" 36+	" 56+	" 181++	Dec. 6. L. adrenalectomy; phosphoric acid. Feb. 27, 1917. Thyroidectomy.
					1917	*1917*				
15	4–86	75	790	"	Feb. 27	Feb. 27	R. 44++ L. 44++	R. 63+++ L. 63+++	R. 197++++ L. 197++++	Feb. 27. L. adrenalectomy; KOH. May 1. Thyroidectomy.
					1916	*1916*				
16	4–26	83	1,620	"	Dec. 5	Dec. 5	" 38++	" 290+++	" 1,181+++	Dec. 5. L. adrenalectomy; phosphoric acid; double ligation of vas deferens. Apr. 5, 1917. L. gonadectomy; partial thyroidectomy.
17	4–25	83	1,450	F.	" 5	" 5	" 38+	" 84+++	" 185+++	Dec. 5. L. adrenalectomy; phosphoric acid; partial thyroidectomy.
					1917	*1917*				
18	4–110	84	1,050	"	May 8	May 8	R. 31+++ L. 31++	R. 141++++ L. 141++++	R. 679+++ L. 679+++	Pregnant; thyroids normal.

TABLE II—*Concluded.*

Rabbit No.	Series No.	Age.	Weight.	Sex.	Date of splenectomy (complete).	Date transplanted.	First examination.	Second examination.	Final examination.	Additional data.
		days	*gm.*		*1917*	*1917*				
19	4–112	84	1,090	M.	May 8	May 8	R. 31+ L. 31+		R. 141+ L. 141+	Thyroids normal; lack of growth of transplants; large accessory spleen.
20	4–113	84	1,225	"	" 8	" 8	R. 31++ L. 31++	R. 141+++ L. 141+++	R. 475++++ L. 475++++	Example of acute enlargement of spleen transplant with pneumonia. White blood corpuscles 30,800.
21	4–94	90	1,195	"	Mar. 22	Mar. 22	R. 77+ L. 77+		R. 175++ L. 175++	Mar. 22. L. adrenalectomy.
22	4–95	97	1,200	F.	Apr. 3	Apr. 3	R. 64++ L. 64++	R. 176+++ L. 176+++	R. 1,062+++ L. 1,062+++	
23	4–97	104	1,265	M.	" 10	" 10	R. 58+++ L. 58+++		R. 169+ L. 169+	Spleen much enlarged at time of removal; evidence that transplants grew more rapidly, then decreased with recovery of animal.
24	4–103	111	1,275	"	" 17	" 17	R. 51++ L. 51++		R. 163++ L. 163++	
25	4–119	132	2,240	"	June 7	June 7			R. 111+ L. 111+	Failure to obtain growth with spleen removed.
26	4–77	167	3,025	"	Feb. 15	Feb. 15	R. 56+ L. 56+	R. 75+ L. 75+	R. 209+ L. 209+	Feb. 15. L. adrenalectomy; KOH. May 1. Thyroidectomy.

27	4–78	167	3,445	M.	Feb. 15	Feb. 15	R. 56–		R. 113–	Feb. 15. L. adrenalectomy; enlarged thyroid lobes. Failure to take probably due to error in technique.
28	4–84	173	2,575	F.	" 22	" 22	" 49+ L. 49+	R. 68+ L. 68+	" 106+ L. 106+	Feb. 22. L. adrenalectomy; KOH; thyroidectomy.
29	4–85	173	3,425	"	" 22	" 22	R. 49+ L. 49+	R. 68+	R. 202+	Feb. 22. L. adrenalectomy; KOH.
30	4–79	320	4,835	"	" 15	" 15	R. 56++ L. 56++		" 61++ L. 61++	Died; pneumonia.
31	4–80	320	4,435	"	" 15	" 15			R. 23+ L. 23+	" "
32	4–83	320	2,940	M.	" 20	" 20	R. 51+		R. 107+	Feb. 20. L. adrenalectomy; thyroidectomy. Apr. 3. Gonadectomy.
33	4–68	396	2,525	"	" 8*	" 8	" 33+ L. 33+	R. 114+ L. 114+	" 216+ L. 216+	June 22, 1916. R. adrenalectomy. Oct. 10. L. gonadectomy. Apr. 11, 1916. Thyroidectomy.
34	4–71	396	2,790	"	" 8	" 8	R. 33+ L. 33+		R. 118+ L. 118+	Feb. 12, 1916. Thyroidectomy. Nov. 3. R. adrenalectomy.
35	4–72	586+	3,150	"	" 8	" 8	R. 33+ L. 33+		R. 118+ L. 118–	Apr. 5. Gonadectomy. Oct. 25, 1915. Thyroidectomy.

*Small accessory spleen left.

TABLE III.

Autotransplantation of Spleen.

Rabbit No.	Series No.	Age.	Weight.	Sex.	Date of splenectomy.	Date transplanted.	First examination.	Second examination.	Final examination.	Additional data.
		days	*gm.*		*1917*	*1917*				
1	4–92	52	720	M.	Mar. 20. ¼ removed.	Mar. 20	R. 80++ L. 80++		R. 177+++ L. 177+++	Mar. 20. L. adrenalectomy. Thyroid normal.
2a	4–93	52	700	F.	Mar. 20. ¼ removed.	" 20	R. 52++ L. 52++		R. 177+++ L. 177++++	Mar. 20. L. adrenalectomy. Thyroid normal.
b					Apr. 17. ¼ removed.	Apr. 17	R. 52++		R. 150++	
3a	4–90	52	635	M.	Mar. 20. ⅛ removed.	Mar. 20	" 80+ L. 80+			Mar. 20. L. adrenalectomy.
b					June 9. Remainder removed.				" 177++++ L. 177++++	Very little growth in first 80 days; marked after removal of spleen; compensatory.
4a	4–88	64	1,050	"	Mar. 20. ⅛ removed.	Mar. 20	R. 49+ L. 49+			Mar. 20. L. adrenalectomy.
b					May 8. Remainder removed.			R. 79+++ L. 79+++	R. 177+++ L. 177+++	Thyroid slightly enlarged; very little growth in first 80 days; marked after removal of spleen.
5a	4–89	64	985	"	Mar. 20. ⅛ removed.	Mar. 20	R. 49+ L. 49+			Mar. 20. L. adrenalectomy.
b					May 8. Remainder removed.			R. 79++ L. 79++	R. 177+++ L. 177+++	Thyroid vascular; slightly enlarged. Rapid growth after removing spleen.

6	4–104	80	960	M.	Apr. 17. ⅛ removed.	Apr. 17	R. 53+		R. 163+	No growth with spleen intact.
7	4–105	80	1,055	"	Apr. 17. ⅛ removed.	" 17	" 53+		" 196+	No growth with spleen intact.
8	4–106	80	1,030	"	Apr. 17. ⅛ removed.	" 17	" 53+		" 163+	No growth with spleen intact.
9	4–114	84	1,260	F.	May 8. ⅛ removed.	May 8	" 31+ L. 31+		" 141+ L. 141+	No compensatory growth of transplants.
10	4–115	84	1,075	"	May 8. ⅛ removed.	" 8	R. 31+++ L. 31++		R. 141++ L. 141+	Thyroid vascular and enlarged.
11	4–107	87	1,110	"	Apr. 24. ⅛ removed.	Apr. 24	R. 52+ L. 52+		R. 163+ (very small). L. 163+ (very small).	Failure to obtain growth without spleen removal.
12	4–108	87	1,010	M.	Apr. 24. ⅛ removed.	" 24	R. 52+		R. 198+	Failure to obtain growth without spleen removal.
13	4–96	97	1,245	"	Apr. 3. ¼ removed.	" 3	" 64+ L. 64+		" 176+ L. 176+	No compensatory hyperplasia.
14	4–98	104	985	F.	Apr. 10. ½ removed.	" 10	R. 58+ L. 58+		R. 169+ L. 169+	Decreased with recovery of animal. Partial removal failed to produce growth.
15	4–118	124	1,625	M.	May 17. ⅛ removed.	May 17	R. 21+ L. 21+		R. 132+ L. 132+	Failure to obtain growth of transplant with spleen intact.

spleen removed, was only + at the first examination 80 days later. The remainder of the spleen in this rabbit was then removed and at 177 days the transplants were ++++, while the first two which did not have the remainder removed were only +++ at 177 days. The same reaction is seen in Experiments 4 and 5, little if any growth occurring until the spleen was removed, when a marked growth promptly took place. The effect of splenic deficiency in stimulating the growth of transplants is striking, as is shown either by comparing animals of similar ages with and without partial removal or by comparing the growth of the transplants before and after total splenectomy in the same animal. The stimulus for this increased growth must be chemical in nature and must operate through the blood stream. The compensatory hyperplasia of the stump following partial removal of the gland cannot be separated from a possible nerve influence. The method of transplantation clearly demonstrates that specific nerves are not necessary for this reaction. Some investigators have not been able to obtain compensatory hyperplasia even of the stump *in situ* following partial removal, or of the thyroid, whose functions so far as we know cannot be assumed by any other tissue, and have doubted its occurrence, while others have no difficulty in demonstrating the effect even in transplants. In the work of Halsted[10] and Hunnicut[11] on the thyroid their failure to obtain compensatory hyperplasia was probably due to their failure to induce a thyroid insufficiency, in the production of which two factors of the utmost importance are involved; *viz.*, the amount of thyroid removed and the presence of available iodine. Loeb[12] recently published his results on compensatory hypertrophy of the thyroid following partial removal in guinea pigs. He found that compensatory hypertrophy occurs, though it is necessary to remove nearly all the gland. We have found that in rats, rabbits, and guinea pigs it is necessary to

[10] Halsted, W. S., Hypertrophy of the thyroid gland. Revision of experiments made 25 years ago, *Proc. Soc. Exp. Biol. and Med.*, 1912–13, x, 111.

[11] Hunnicut, J. A., The absence of hyperplasia of the remainder of the thyroid in dogs after piecemeal removal of this gland. Auto-transplantation of the thyroid in partially thyroidectomized animals, *Am. J. Med. Sc.*, 1914, cxlviii, 207.

[12] Loeb, Leo, Studies on compensatory hypertrophy of the thyroid gland. I, *J. Med. Research*, 1919, xl, 199.

remove relatively more thyroid in order to obtain compensatory hyperplasia of the remaining stump than in cats and dogs, and in all cases available iodine must be excluded because of its inhibitory effect.[13] We believe that Loeb would have obtained more constant results had he in all instances allowed an interval of 30 days for compensatory hyperplasia to take place.

Histology of the Transplants.—This has been described elsewhere[1] and only brief mention of certain features need be made. Many of the transplants have reached 5 or 6 mm. in diameter (intracapsular measurements). They have all the general characteristics of normal spleen, both as to the number of component structures—capsule, trabeculæ, lobules, Malpighian bodies, pulp, sinuses, blood pigment—and their relation to each other. No attempt has been made to demonstrate the presence or absence of smooth muscle fibers in the capsule and trabeculæ. Apart from this the spleen is capable of complete regeneration. These studies indicate that while anatomically the spleen is very complex, biologically all the major elements are simple and endowed with uniform and marked regenerative capacity.

Permanence of Spleen Grafts.—Most of the experiments were terminated within a year. Two rabbits (Nos. 16 and 22) have been allowed to survive and at the examination on March 1, 1920 the transplants in each were found active and very vascular—1,181 and 1,062 days respectively. They are possibly slightly smaller than at the second examination 290 and 176 days after transplantation. Both rabbits were young at the time of transplantation and in both complete splenectomy was performed, thus insuring growth of the transplants. Both rabbits are still strong and active. One can conclude, therefore, that spleen autografts made under conditions which insure good initial growth are permanent. There appears, however, to be a slight involution or atrophy with age even in splenectomized rabbits, and, as already pointed out, transplants made in old rabbits without splenectomy may in time (several months) undergo complete atrophy. Our experiments with autotransplan-

[13] Marine, D., and Lenhart, C. H., Colloid glands (goiters): their etiology and physiological significance, *Bull. Johns Hopkins Hosp.*, 1909, xx, 131.

tation of the spleen and also of the thyroid show that at least an anatomical deficiency is not necessary in order that transplants may take and remain active for several months. Growth of these grafts, however, usually does not occur unless there is a physiological insufficiency which may exist independent of the amount of functionally active organ. Loeb and Hesselberg[14] have also shown that the taking of transplants is independent of a physiological insufficiency. Halsted,[15] working with the parathyroid, concluded that it was necessary to induce a physiological insufficiency in order to obtain successful transplants.

Reaction of the Grafts in Acute Infections.—No experiments have been made relative to this point. In a few instances in which the rabbits died of pneumonia, the transplants were markedly congested, and in one rabbit (Experiment 19) which died of pneumonia, the transplants at autopsy were soft, engorged with blood, and microscopic examination showed increase in pulp cells. In the ordinary sporadic cases of pneumonia in rabbits the reaction of the spleen is so variable and even in healthy rabbits there are such variations that it would be necessary to carry out a series of experimentally controlled infections to obtain definite data.

SUMMARY.

No instance of survival of spleen homeografts beyond the usual taking and persistence for 1 or 2 weeks common to most homeografts has been observed, although the possible advantages of consanguinity, age, and splenectomy were fully utilized. This is in sharp contrast to thyroid, sex gland, and adrenal cortex homeografts, with which one may expect 10 per cent to survive the 30 day period. It suggests that spleen is a stronger antigen and excites a greater degree of immunity more quickly. With autografts survival and growth are the rule, and failures are due to technical errors. Age is an important factor in the growth of autografts. The younger the rabbit the

[14] Loeb, Leo, and Hesselberg, C., Studies on compensatory hypertrophy of the thyroid gland. II, *J. Med. Research*, 1919, xl, 265.

[15] Halsted, W. S., Auto- and isotransplantation, in dogs, of the parathyroid glandules, *J. Exp. Med.*, 1909, xi, 175.

more growth is aided. This beneficial effect decreases gradually and becomes negligible after sexual maturity. Removal of the spleen is a powerful stimulus to the growth of transplants. The effect varies inversely with the age and usually is negligible after sexual maturity. The influence of age and splenectomy suggests that the spleen is most important in early life and after sexual maturity is either unimportant or its functions may readily be assumed by other tissues (hematopoietic). Anatomically the spleen is a highly complex structure, but biologically all the major elements of the spleen are simple as indicated by the uniform and marked regenerative capacity. There is a tendency for grafts to involute or atrophy with age, and grafts made in old rabbits without removal of the spleen may undergo complete atrophy. Grafts made in young rabbits, accompanied by splenectomy, have been observed for more than 3 years and may be said to be permanent. There is some evidence that subcutaneous autografts react to infections in the same way as the intact spleen.